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Society of University Surgeons

TO CONTRIBUTE RICHLY TO SURGERY

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TWENTY years ago, when the Society of University Surgeons was 2 years old, Dr. Owen Wangensteen, then as now Chairman of the Department of Surgery of the University of Minnesota Hospitals, and professor in the medical school, wrote an editorial which appeared in SURGERY. The title was "The Society of University Surgeons and the Need for a Surgical Forum." It described the founding of this Society, its objectives of encouragement of clinical and laboratory research by its members, the development of methods of graduate teaching with particular reference to the resident system, and free and informal interchange of ideas on these subjects by a limited membership with common aims. Dr. Wangensteen commented on the need for a forum in surgery for young men since new ideas came in the main from young and creative minds, and concluded with these words: "If this or any other surgical group satisfies the pressing need for a surgical forum before which the most creative thought and original achievement in American surgery can secure an audience, it will fill a long-felt want, exert a large and important influence, and contribute richly to surgery."

It seems appropriate, 20 years later and on the occasion of the Society's meeting in Minneapolis, which is the home of 14 of our members and the training ground of several more, to look at some of the problems that confront us today in the light of the challenge to become "an important influence and contribute richly to surgery."

The Society's founding members defined the object of the Society as "the advancement of the art and science of surgery by the encouragement of its members to pursue original investigations both in the clinic and in the laboratory, the development of methods of graduate teaching in surgery with particular reference to the resident system, and free and informal interchange of ideas pertaining to the above subjects as a limited membership and common aims make possible." Usually, the papers presented before the Society have been concerned with research, while presidential addresses have been

Presidential Address read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

concerned with various aspects of the residency system and of the teaching of surgery at the graduate level. In 1954, Dr. J. Englebert Dunphy reviewed in his presidential address the history of the Society of University Surgeons, and in a delightful and penetrating manner defined the university surgeon. This address is recommended to all of you who have become members since that time, both for its historical value and for its pertinence today in defining the surgeon-teacher-investigator who is, or should be, the university surgeon.

In the past few years the Society has become concerned for the effect of its own growth upon its objectives and particularly for the inverse ratio of size to informality and freedom of discussion. It is also becoming more and more aware of strong and changing social and economic forces which are altering many aspects of medicine in ways which seriously threaten the residency system and surgical research as we know them today. As Dr. C. R. Hanlon, in his scholarly address last year, pointed out, scientific societies cannot live in a vacuum, their decline and fall are due either to their too limited or untimely objectives, or to their inability to adjust to changing times, or both. The vigor of this Society, as created by the interest and enthusiasm of its membership and its ability to date to surmount major problems, makes me optimistic for the future, but I am equally sure that if our objectives are to be continued as we wish them to be in the future, we as a Society and as individual members of it must assume responsibility in reaching out beyond the narrow and pleasant confines of our own company. As individuals, we must continue and expand participation in organized medicine at local, regional, and national levels. We tend to criticize the activities of the A. M. A. and of state and county societies, while we busy ourselves within our ivory towers and allow our voices, our votes, and often even our memberships to go by default. As a Society we must consider studying carefully and taking a position on major issues that affect us, lest again by default our voice is unheard where decisions vitally affecting our interests are being made.

Twenty years ago, when the founders group of the Society of University Surgeons gathered for the Second Annual Meeting of the Society, Dr. Samuel Stabins of Rochester, New York, who conceived and carried out the idea of there being this kind of surgical Society, and who was its first president, spoke at the annual dinner on the subject of "The Resident Surgeon," tracing the concept of the surgical residency from Halsted to that time. Five new member departments of surgery were admitted, and a total of 50 members were elected from the then 15 member Departments, making the total membership 60. Retirement to senior membership on reaching the age of 45 had already been decided upon, as had the object of the Society of advancing the art and science of surgery through research by its members, study and development of graduate surgical training, and a limited active membership. The average age of those elected to membership that year was 36.1 years, giving them an average active membership of 9 years. This is of some interest since in recent years men elected to membership have averaged 37 years in age, and have had an expectancy of 8 years of active membership. This year's new members average 35.8 years of age.

Membership in the Society grew slowly during the war years. A total of 38 men were admitted from 1941 through 1944, inclusive, and the total member departments of surgery were increased to 22. There was no meeting in 1945, since by this time more than half the members were in military service.

In 1946, the 45-year age limit for active membership began to take its toll of the founding group, and by 1950 Dr. Jean Stevenson, then President of the Society, became a senior member, the last of the founders group to do so.

In the interval, major changes in policy of the Society were effected by amendments to the constitution which permitted senior members the right to vote while denying to them the right to hold office, and the Lockwood Amendment was passed which opened membership in the Society to candidates not graduates of member surgical department residency programs, if they met the simultaneous qualifications of a training of high standard and a "continued productive interest in academic surgery," with the academic rank of Assistant Professor of Surgery, or a degree of attainment considered equivalent thereto by the Executive Council.

Even with these changes, the Society continued to elect a varying number of candidates, usually well below the maximum permitted, and the constitutional limit of active members was never achieved. In the years 1946 through 1950, inclusive, 65 men were elected. In the years 1951 through 1955, 80 members and 5 honorary members were elected. In 1954, the limit on the number of men who could be elected in any 1 year was raised from 20 to 25.

In the past 4 years we have elected 82 new members, achieving the constitutionally permitted limit of 25 for the first time last year.

At the end of the meeting of the Society in 1957, a quiet milestone was passed when the number of living senior members exceeded the active members for the first time. Last year the figure had risen to 172 senior members compared with 135 active, with 15 honorary members not included in the totals. If my arithmetic is correct, the roll of senior members was increased this afternoon by 22, including your speaker, thus giving us 193 senior members. A maximum possible slate of 25 candidates, if elected, would bring the active roll to 159. Since the average life expectancy of an American male at age 37 is 39.5 years, according to the U.S. Public Health Service statistics, it does not take much mathematical juggling to figure out that our average university surgeon has 8 years of active membership and 32 years of senior membership ahead of him, and that our Society, which began its life with and still holds to the concept of a limited membership and informality of exchange of ideas, has at present about 350 members of whom nearly 200 are senior members. If we increase the yearly maximum elected to 30, as I believe we should in order to provide membership for more of the outstanding candidates proposed by our own members, we face in not so many years a senior membership of between 700 and 800, outnumbering the active membership by nearly 4 to 1!

It is apparent that this is no longer a small society, and that it will inevitably grow larger in total membership.

I am convinced that if the Society of University Surgeons is to remain a forum where the creative, young, idea-filled minds of surgery are to be heard, and if the Society is to achieve its maximum usefulness in the future in representing the *young* university surgeon in a changing world, the senior members had better be along just for the ride, as Bert Dunphy once described it, and that the driving should be done by the active membership as far as the policies of the Society are concerned. I would hope to see favorable action in the near future on an amendment to the constitution which would exclude the top-heavy senior membership from any chance of dominating policy within the Society, and pledge you my vote to this end if future councils recommend it.

The problem of numbers does not end with members and senior members. Each member of the Society, before he is elected, has shown promise in the sense of having contributed significantly to the surgical literature in addition to having convinced his sponsors of his potential. With three to four times the maximum number of candidates permitted for election nominated and seconded and strongly supported by members of this Society, and with the entire list submitted to the membership for comment prior to Council review, the task of the Council is still an exceedingly difficult one. Each man recommended for membership must have passed the rigid tests of originality and significance of his contributions, as well as showing productivity. Presumably, if the Council has chosen well, each man recommended for membership and elected by the Society will continue to show originality and significance in his work, and will wish to present papers before the Society. With the scientific program from the membership limited to 23 papers, many of them of 10 minutes in length, and with the program open to abstracts from all of the membership, it is not difficult to see that the membership can be represented on the program an average of *once in 10 years!* Last year 111 abstracts were submitted and 23 were chosen, the preceding year the total submitted was 118. The quality of these abstracts is so high in most instances that the program committee is hard put indeed to select a program, and many members must receive the letter which begins, "We regret to inform you. . . ." The quality of the work is attested by the fact that virtually all of the papers rejected appear in surgical journals in the following year.

The Society should consider ways in which this problem can be ameliorated. One way would be to increase the scientific sessions by the membership by a day, as is now the case with the American Surgical Association. This would have to be at the sacrifice of the host department's program, or would mean an absolute increase in the length of the meeting. I personally do not favor either of these alternatives. The program of the host institution provides a real perspective of the activities of the department being visited, and an opportunity for young surgical investigators to be heard by a critical and appreciative audience before qualifying for membership. The extension of

our meeting beyond 2½ days, with travel time added, would seriously reduce the ability of many to attend, or would result in attendance for one or another day to the detriment of the continuity as well as the fraternity of our Society.

It would seem wise to consider the possibility of 2 simultaneous scientific sessions, at least for part of the program, in order to accommodate additional papers. These could be arranged by subject matter in such fashion that a minimum of conflict of interest would result for most members. Combined general sessions should be held as well at which papers of general interest or outstanding significance could be presented. It would seem reasonable for at least a portion of the papers selected for a meeting that the abstracts submitted by or including the name of an active member of the Society as a coauthor should be given priority.

Still another problem of direct concern to the Society which should be considered is that of the category of member departments of surgery. Until the Lockwood Amendment became effective, membership in the Society of University Surgeons could be achieved only by men who were resident graduates of member hospital programs who, subsequent to completion of their residency programs, had occupied a faculty position in a university medical school and its affiliated hospital for 18 months. The Lockwood Amendment, which permits the Society to choose a man for what he is, rather then requiring a relatively restricted background as a start, has freed the Society to attract outstanding men however they may have come into academic surgery. It is probably significant that only one member department of surgery has been admitted to the Society since 1955, and that the number of inquiries has been relatively small until this year.

The responsibility for accreditation of surgical residencies is now firmly in the hands of the Joint Conference Committee on Post-Graduate Education in Surgery. A tri-partite creature of the Section on Surgery of the A. M. A., the American College of Surgeons, and the American Board of Surgery, this Committee is administered by the Council on Medical Education and Hospitals of the A. M. A., and bears the prime responsibility for investigation and approval of residency training programs in surgery. As of October, 1959, there were 704 approved programs in general surgery offering 5,837 residency positions in all years, and 2,201 in the first year. More than half of these programs are in non-university-affiliated hospitals. This broad type of approval, although vital in providing surgical training for many men to serve a large and rapidly expanding population, is not in itself intended to require the type of program associated with university surgery. There seems, then, to be a place for *standards for surgical departments of university hospitals,* and for the advice that an organization of university surgeons can give to departments striving for this type of excellence. Our bylaws provide 2 basic requirements of departmental membership; first, that the residency program of the department provide (1) undergraduate teaching, (2) facilities for, and adequate stimulus toward, investigation, and (3) wide responsibility for patient care in the whole final year of the residency, and, second, that the

department will have produced, through its residency, an individual who has been admitted as a member of the Society of University Surgeons.

The Committee on Graduate Training has been considering this problem actively this year and by the time of this address may have produced some definitive recommendations. I personally urge that the member department concept be continued, be strengthened by required review of departments not producing new members within a stated period, and that other university hospital surgical departments be encouraged to qualify.

To this point, this discussion has been concerned with problems of the Society of University Surgeons which have arisen within the Society mainly as the result of its growth, and of changes in policy in the past 22 years. These problems are essentially soluble by the Society from within, or within the framework of the Society's close relationship through membership and tradition with university departments of surgery in the United States and Canada. However, one of the major interests of the Society, graduate training in surgery with emphasis on the residency system, is facing today the most serious crisis that has been faced since World War II disrupted all training programs. It is one which, in my opinion, seriously threatens the concept of the graded and progressive responsibility of residents through 3, 4, or more years of training culminating in wide responsibility for patient care in the senior year, and threatens to put us back many years to the preceptorial type of surgical training which preceded the development of residencies, and is still common in many parts of the world today.

The problem is, of course, that of the vanishing service patient, the result of rapidly expanding health insurance plans of all sorts, and of the general national prosperity. This Society is well aware of the problem, having considered it of sufficient gravity by 1955 to pass a resolution, proposed by Dr. Clarence Dennis, requesting the active aid of the American College of Surgeons in attaining a solution of the problem of utilizing insurance cases for graduate surgical teaching.

In 1957, the presidential address of Dr. Harold Zintel was devoted to "The Impact of Health Insurance on Surgical Training." He pointed out that in 1940 approximately 9 per cent of the population held some type of voluntary hospitalization insurance, and that by 1956 66 per cent of the population had hospitalization insurance of some sort, and 56 per cent had some form of insurance for surgical fees. In addition, he emphasized the fact that medical education and research stand alone among major industries and services as an activity in which the cost of research and training is not passed on to the consumer. He suggested that the desperate economic plight of many medical schools and their affiliated hospitals might be met at least in part if the fees for professional services available through insurance on patients, who otherwise would surely be of ward status, could be collected and used to help defray the cost of the educational program. He suggested also that $100 per year, sent from each doctor in practice to his medical school, would not in 20 years even repay the costs of his education alone, but

it would provide $20,000,000 per year toward medical education which would be 4 times that now provided by combined totals of the American Medical Education Foundation, the National Fund for Medical Education, and Voluntary contributions by the profession to individual medical schools.

The gravity of the problem of diminishing service patient ratios in a university hospital is illustrated in a paper given last year by Dr. Herman E. Pearse as a part of the proceedings of the Fifty-fifth Annual Congress on Medical Education anl Licensure. He showed that at the Strong Memorial Hospital in 1934, when Blue Cross started, 68 per cent of the patients were in the clinic category. By 1940, this number had fallen to 31.2 per cent, and by 1948, to 8.6 per cent. The addition to the ward population of those patients who had hospitalization insurance only, but were otherwise medically indigent, or who chose to go on the ward service, raised the ratio to 20 per cent in 1950, but the advent of Blue Shield, providing a fee, has resulted in a further decline to 12 per cent in 1958. Were it not for the adjacent municipal hospital, the adequacy of clinic patients for surgical residency training would be in serious question.

An approach which has proved useful is the affiliation of university hospitals, often voluntary, nonprofit corporations, with municipal hospitals, to their mutual benefit in providing indigent patient material and providing university department of surgery standards of supervision. Affiliation with smaller community hospitals with rotation of residents, as described by Fred Ross to this Society in 1957 in a symposium on surgical teaching, is another. Still a third source of patients is composed of those in Veterans Administration Hospitals and Public Health Service Hospitals. Dr. Ravdin has suggested that these institutions should play an increasing role in surgical residency training, and many are now affiliated with university programs throughout the country.

A fourth possible source of patients for training of residents is municipal and state institutions for the insane, and penal institutions. A discussion of the use of these types of institutions for affiliation in training has been presented as a part of the Panel on Surgical Education at this meeting of the Society.

However, each of these sources of clinical material suffers from the common fault of decentralization. Few university hospitals are fortunate enough to have a municipal hospital across the street, and distance reduces the close and continuing contact between attendings and residents, and among residents on different services, which is an important part of learning. When institutions for the insane or penal institutions are used the residents assigned are often quite isolated during their service. Veterans Administration Hospitals and Public Health Hospitals quite properly have their own training programs, with Chiefs of Surgery who are not always appointees of the Chairman of the University Department of Surgery, and conflicts of interest may develop. The Veterans Hospitals have the added disadvantage of a skewed surgical experience with older male patients predominating.

There is no doubt that all of these avenues should be explored and utilized as fully as possible, consistant with maintenance of residency standards, but it is not the ultimate answer in my opinion.

Many of you will recall the panel on the problem of service patients which was held at our meeting last year in Denver. This is reported to be in the process of publication, and should be published because some of the conflicts of opinion and objectives that exist within medicine were sharply brought into focus that day. Dr. Zintel, outlining the problem, pointed out the increase in insurance coverage (including Blue Shield), stated that Blue Shield in many areas had refused to pay duly licensed residents for services they performed, and that the medical societies had permitted this to happen. He went on to say that the effect of insurance on training of qualified surgeons and the simultaneous financial plight of medical schools and teaching hospitals in a time of unprecedented national prosperity represented serious challenges to our present system of medicine.

The representative of Blue Shield for the State of Colorado pointed out that the responsibility of insurance agencies was to indemnify their contract holders against medical and surgical expenses, and that their policy was set by their directors, a majority of whom are physicians.

The representative of a union made the union position perfectly clear. They want board certified or eligible surgeons to take care of union members, in a manner which satisfies the union members, at a cost which the union can afford and, by implication, may establish. He stated that the union could not afford to support teaching or research, or pay for too many laboratory tests or too long a hospital stay. He made certain suggestions which deserve serious consideration. The first is that he recognized university residency training as the best type of surgical training, and would accept sufficiently qualified and supervised resident operating. He suggested that university hospitals consider expanding their outpatient departments in cooperation with the unions and union health plans to care for union members. It was implied that the facilities and the attitude toward indigent patients that characterized some municipal hospitals would not be satisfactory to union members, and that teaching hospitals would have to compete in services and facilities and cost as well as professional care.

There are 2 things which, in my opinion, we as university surgeons can and must do if the surgical residency as we know it now is to survive. The first of these is to compete strongly and with the conviction that what we have to offer is the best kind of surgical care that patients can get. The second is to take a more active role in the organizations which determine policy in medical affairs in our communities and states, and bring to these bodies the problems of the university medical schools and the teaching hospitals.

With respect to competition, Dr. Ward Darley, Executive Director of the Association of American Medical Colleges, and Dr. Gardner Child have both spoken to the major point. With 70 per cent of our population now covered by hospitalization insurance and several millions more protected by

the Veterans Administration, Military Services, Medicare, and union plans, and with more to come with the probable entry of Social Security into the medical care field, most of our citizens will be assured of the right to choose their own health and medical care among a wide spectrum of choices, and virtually all will be assured of some arrangement which prevents them from being charity cases.

This being the case, if teaching services are to survive as they must in the best interest of the public and of medicine, then institutions organized for teaching and research must be included in the choice which the patient has when he is protected by an adequate medical care plan. There is a clear challenge here, for if patients are *to elect* to be admitted to a teaching hospital and on a teaching service, the teaching service must emerge in the public mind as clearly superior to what is available to them otherwise. To achieve this will require a concerted and continuing effort in public education on the part of the medical schools and teaching hospitals and by the staffs of these institutions, who must believe in what they are doing.

The surgical resident must stop being treated as a second class doctor by the profession and in the public eye. He should first of all be licensed to practice medicine in the state in which he is taking his training, at least by the time that he begins to perform surgical operations himself, and the fact that he is licensed and is a member of the surgical team of the teaching service should be made readily available so that it becomes common knowledge in the community. Many surgical residents today hold rank as assistant or instructor in the department of surgery of the medical school with which their hospital is affiliated. This rank reflects their responsibility in teaching medical students. Similarly, it would seem quite in order for them to hold junior rank in their hospital during the period when they have substantial responsibility for patient care, this rank being a reflection of their responsibility in precisely the same manner as their university appointments.

Patients who come to the teaching hospital as paying patients, that is, those with the ability through insurance to pay for professional services, but who are not referred to specific attendings as private patients, would then be assigned to assistant members of the surgical team by the senior attendings in accordance with the assistant's experience and ability to handle the particular problem. Consultation with, and supervision by, senior attendings would be an intrinsic part of patient care on teaching services, as it is today. The sharp distinction between "charity" and private cases, and between resident and attending, are both modified by inevitable circumstances.

It would be presumed that quarters and facilities the equivalent of semi-private facilities would be provided by the hospital for these patients who might come to the hospital as individuals, or through contracts for medical care established with organizations such as industrial corporations or labor unions.

The few truly indigent patients who require care would be assigned to the junior staff in precisely the same fashion as above, and as is the case today.

Two important limitations are necessary if this type of arrangement is to be successful. The first is that the teaching hospital's teaching services shall be limited in size and in the number of patients handled by the resident staff to what is actually required for the teaching program, and, second, that the income derived from the practice of the resident assistants, who should be on a full-time basis, should be handled by some form of professional group established for this purpose, and should be used only for professional teaching salaries, direct teaching functions and costs, and research of interest to those who are actively participating in the teaching program.

Is this not a better, more clean-cut, and more honest method of meeting a major challenge of the 1960's than the infinite variety of subterfuges which have arisen and are in variable use throughout the country today to permit enhanced surgical experience by surgical residents in a wide variety of hospitals?

This plan does not take into account the foreign-trained doctors, some 1,233 of whom in 1959 were in residency programs in the United States. Their programs would of necessity have to be different since many cannot meet the licensure requirement. In general a moderate number of foreign-trained doctors, with satisfactory basic training as determined by examination, could continue to be trained in the United States in programs where they do not have to assume direct responsibility for paying patients.

Of necessity the approval of the Joint Conference Committee on Post-Graduate Education in Surgery and the American Board of Surgery must be secured for a total training experience as offered by teaching hospitals under this or similar plans. Since the concern of both organizations is with the quality and product of residency training in surgery in the United States, and since both groups are keenly aware of the diminishing surgical experience in many well-organized and excellently conducted surgical residency programs, it would seem to be a matter of evolving the details if the voluntary, nonprofit type of university hospital is to survive as a teaching center.

There remains one exceedingly important and indeed vital aspect of this problem to be resolved, and that is for the medical schools and the teaching hospitals to seek and achieve the understanding and the cooperation of organized medicine in maintaining high-quality graduate education in medicine. In our failure to appreciate the need for mutual understanding, and to seek help from our colleagues at the local and state levels in organized medicine, we of the University hospitals have in my opinion been remiss. When economic factors such as insurance fees become the dominant factor in discussion, and when the faculties of medical schools and the staffs of teaching hospitals fail to participate actively in the affairs of county and state societies, as is too often the case, mutual misunderstanding and mistrust are the inevitable result. In the instances with which I am familiar, where Blue Shield plans have refused payment to residents for services rendered to insured patients, this kind of atmosphere exists. Where problems and points of view have been and are freely discussed and understood, and where the faculties

of medical schools and teaching hospitals participate actively at the local level in medical society affairs, there is a much better understanding and problems of this type are being worked out.

There is understanding at the level of the Council on Medical Education and Hospitals of the A. M. A., but the problem cannot be solved there. There were, as of Dec. 31, 1958, 83 Blue Cross hospitalization plans with 55,880,414 enrolled, and 72 different Blue Shield plans with 42,575,256 enrolled. Each of these plans is controlled at state or even county level by its professional and lay trustees. The provisions of plans vary considerably from plan to plan, as do their policies. Efforts are being made to develop nation-wide Blue Shield plans with similar provisions throughout the country. Nation-wide industries and nation-wide labor unions, both powerful forces in the support of health plans, are dissatisfied with piecemeal decisions, varying from region to region or even community to community, and are putting great pressure on the plans for uniformity. The needs of medical education are surely even more urgent and require national understanding by the public as well as the insurance carriers.

At the present time, however, these plans are controlled at local or regional level, and it is at this level that understanding must be sought. It becomes encumbent then upon the members of this Society, concerned as we are with graduate training in surgery, to take action as some already have, at local levels by bringing to county and state medical societies the urgent dual problems of the financial needs of medical schools and of teaching hospitals, and the vanishing service patient as what they are—serious threats to medical education.

It seems evident from personal experience that most of our colleagues are almost totally ignorant of the problems involved. A vocal minority, often in prominent positions in local societies, may make life difficult at times, but the average doctor is willing to listen to a reasonable and factual presentation, and once he understands is willing to help, or at least not be opposed to what must be done to meet the crisis in postgraduate education that exists today.

It seems entirely reasonable that if county, state, and national medical society members understand the present problems, and if the profession can be assured that the competition of the teaching hospitals' resident teaching services is limited to the number of patients, and no more, that are necessary to accomplish proper teaching of medical students, interns, and graduate physicians who are specializing, much of the fear and jealousy that motivate many today will be dissipated.

The following general points would seem to be a basis for developing understanding and agreement:

1. Complete honesty should be used in dealing with insured patients who come to the hospital in informing them of the alternatives available to them under the terms of their insurance, including additional costs, if any, in hospital charges or professional fees that they may face in electing either private or teaching service care.

2. The "charity" aspect of teaching service care should be eliminated from consideration. These patients are paying their way whether they choose one or the other alternative.

3. They should have clearly explained to them the status in the hospital staff team of the man who is to be their doctor, but the position of the resident or assistant surgeon, whichever he may be called, should not be degraded. After 4 or 5 years of graduate training in an approved internship and surgical residency, and holding a state license, he is hardly an incompetent amateur! The availability and use of senior attending consultation and supervision without cost to the patient are valuable assets to the teaching service as well, and should be included in the patient's information.

4. Clear understanding should be achieved on the collection and use of fees derived from insurance. The basic principles would seem to be (a) that the man performing the service should be licensed, and should collect the fee, (b) that these fees may be collected by men in training status only if they are a part of a fully accredited residency program, and (c) that the fees earned by men in training status should be kept in a fund, separate from general hospital operating funds, and must be used under professional control only for the educational, training, and research activities of the training program.

If these or similar principles can be accepted by the profession as a whole, the future of graduate training in surgery seems assured. It is the obligation of university surgeons, as a part of all those genuinely interested in postgraduate training in surgery, to work toward this goal.

These, then, are some of the problems as I see them which face our Society at the beginning of the 1960's. We have grown large, and inevitably will grow larger as time goes on. We must be prepared to face the problems thus created. We must also be prepared to take an active role as a Society, and as individual members of it, in the problems now coming to a climax of finding sufficient support and suitable patients for proper graduate training at the residency level, and in the national readjustment of thinking that is necessary to accomplish this with paying patients. The members of this Society have wide influence today in surgery. We must face all challenges of this new decade with courage and with conviction if we are to continue to meet the challenge of "contributing richly to surgery."

STUDIES OF THE EFFECTS OF HYPOTHERMIA UPON CEREBROSPINAL FLUID OXYGEN TENSION AND CAROTID BLOOD FLOW

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ALTHOUGH external body cooling has been employed on the Neurosurgical Service of the University of California, Los Angeles, since 1954, our knowledge is incomplete as to what it accomplishes in affording protection to the brain and how such protection is brought about. The present study was undertaken to apply new measuring techniques to the collection of data which may improve our understanding of the effects of hypothermia.

When the body is cooled externally, or when the circulating blood is cooled directly, the cold blood carries heat away from brain tissue, the function of which alters in a fairly predictable fashion. The functional result observed may be caused by (1) direct effects of cooling upon the metabolism of the brain cells and their processes, (2) variations in the rate of chemical and electrical reactions and perhaps the quality of the chemical and physical environment of the cells and their processes, or (3) a combination of both.

The environment of the cells and their processes is influenced mightily by the cerebral circulation. Irrespective of what metabolic activities occur anaerobically, tissue survival depends upon those processes served by cerebral circulation. Measurement of the circulation and sampling of some parameter of the environment of the cell may tell us qualitatively and, at times, quantitatively what is available to the cell and its process. It is only by indirect inference, however, that we can guess what, of the available supply, the tissue uses. If the cells are given only what they need, then our measurements in the environment reflect cell requirements. If the supply of oxygen to the cell is less than that needed and is of sufficient duration in its deficient state, then permanent functional and/or structural changes might be expected from hypoxia. If the supply is in excess of need, this might be difficult to interpret from our measurements.

Investigators have utilized varying techniques and different animals to probe these problems. The present study combines the polarographic principle of oxygen reduction for measuring oxygen availability by the use of the commercially available Clarke-type electrode† and an electromagnetic flow principle implemented by Kolin[8, 9] and Wetterer[18] to measure blood flow. These techniques were applied to an experimental system designed to study the effects of body cooling upon cerebrospinal fluid oxygen availability and bilateral carotid blood flow.

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*Mr. Good was a Blalock Student Summer Fellow in 1958 and 1959.

†Manufactured by the Spinco Division of Beckman Instruments, Inc., Pasadena, Calif.

METHODS

Adult mongrel cats and Cynomolgus or Macaque monkeys were utilized during these studies. All animals were anesthetized with intravenous or intraperitoneal pentobarbital sodium circa 35 mg. per kilogram of body weight. The cats were artificially respired at parameters to produce a mild hyperventilation. The monkeys were rendered apneic by d-tubocurarine in all experiments except those in which spontaneous respiration was desired. The hair of the animals was clipped, and their heads were supported in a stereotaxic apparatus. Cisternal fluid pressures and/or femoral arterial pressures were monitored, as were cerebral hemisphere and esophageal temperatures. Continuous bilateral carotid artery blood flow was measured by the electromagnetic flow instrument of Kolin in the cat studies and by a similar instrument made available through the courtesy of Dr. Nicholas Assali for the monkey studies, in which the metering probes were placed circumferentially about the intact arteries. Oxygen tension of the cisternal cerebrospinal fluid was measured by the commercial adaptation of the Clark electrode incorporated in a rigid sheath in which the platinum cathode is placed in contact with the silver anode via a thin film of electrolyte beneath a polyethylene membrane. The electrode was inserted into the cisterna magna through a thin-walled No. 17 gauge cannula, such that its tip was continuously bathed in fluid. After a stabilization period the animals were bathed in an ice brine at 2° C. The cats were cooled until the heart beat ceased. The monkeys were rewarmed when esophageal temperatures reached the mid-20° C. range. In 2 monkeys, observations were made of the effects of ischemic and anoxic anoxia by bilateral carotid occlusion or the production of apnea. All animals were sacrificed at the completion of the observations.

RESULTS

Cats.—

Temperature: Brain and esophageal temperatures fell concomitantly until temperatures between 28° and 25° C. were reached, below which brain temperature lagged behind esophageal figures. This cooling gradient coincided with an abrupt decrease in carotid blood flow. Terminal esophageal temperatures fell to 22° to 17° C. at which levels the brain lagged 3 to 7 degrees above. Cooling occurred over a time span of 30 to 85 minutes.

Pulse: Except for an initial rise in 3 of 5 animals immediately after immersion in the brine, the heart rate fell as a function of temperature in linear fashion at a mean rate of approximately 5.3 per cent per degree centigrade (Fig. 1).

Blood pressure: After immersion of the cats in the brine, the femoral arterial pressure rose sharply above the precooling mean until temperatures between 33° and 31.5° C. were reached. The mean rise was 23 per cent. Below these temperatures, the pressure fell in linear fashion with temperature at a rate of 6.9 per cent of the precooling base line per degree, but did not actually fall below the base line until temperatures between 30.5° and 23.5° C. were reached. The blood pressure, because of the initial rise noted, was found to be 10 to 30 per cent higher at any given temperature than the corresponding level of pulse or carotid blood flow (Fig. 1).

Carotid blood flow: The precooling base line mean value for 5 cats was 68 ± 34 c.c. per minute. Following exposure to cold, a mean rise of 12 per cent in carotid flow was measured. Between 32° and 31° C. (brain temperature), the flow began to fall and continued at a rate approaching 9 per cent per degree centigrade (Fig. 2, *A* and *B*). The percentage reduction of blood flow below the precooling base levels was greater than in the case of blood pressure (Fig. 1).

Cisternal oxygen tension: The precooling, postanesthesia mean value for 5 animals was 33 ± 11 mm. Hg oxygen tension (34 C. esophagus). As esophageal temperature was reduced to 30° C., the oxygen tension fell gently, and thereafter more sharply (Fig. 3, *A* and *B*). The percentage change from the precooling base line of carotid blood flow and oxygen tension (corrected for brain temperature and esophageal temperature) is charted in Fig. 4, *A* and *B*.

Whereas the general trend of oxygen tension in the great cistern was downward as temperature fell, the marked fall occurred below the temperature of

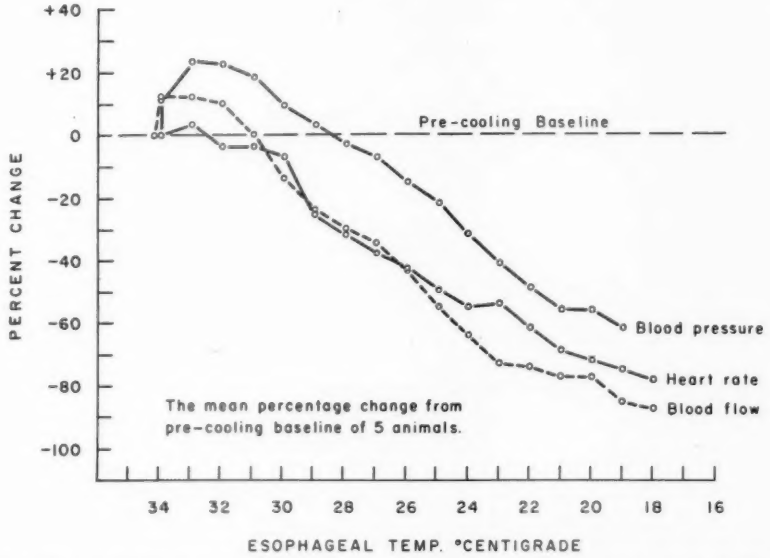

Fig. 1.—The relationship of femoral artery pressure, heart rate, and carotid blood flow to esophageal temperature in cats.

Note the proportionately greater fall in carotid blood flow and pulse rate compared with femoral arterial pressure.

28° C. On the basis of esophageal temperature, blood flow fell more precipitously than oxygen tension until the temperature of 24° C. was reached, beyond which oxygen tension fell rapidly. On the basis of brain temperature, this same divergence was noted to 26° C., below which the curves again closed toward one another.

Cisternal cerebrospinal fluid pressure: Following an initial rise after immersion in the cold brine, the pressure steadily fell to approximately 22° C., at which esophageal temperature the cisternal pressure values were 28 per cent below normal.

Monkeys.—

Temperature: Brain temperatures (cerebral hemisphere) lagged behind esophageal temperatures during both the initial cooling process and the subsequent rewarming process, i.e., the brain cooled more slowly and, once cooled, warmed up more slowly than temperature reflected by the esophageal probe.

Pulse and blood pressure: Both of these items decreased as a function of temperature.

Carotid blood flow: In 4 animals with technically adequate flow data, the mean precooling value for the combined carotid flow was 63 ± 24 c.c. per minute.

Change in posture of the animal's head was often associated with alteration in carotid flow and blood pressure usually in the direction of elevation of both when the animal's head was elevated to the erect posture, provided no curare had been given. The administration of curare preparations sufficient to paralyze respira-

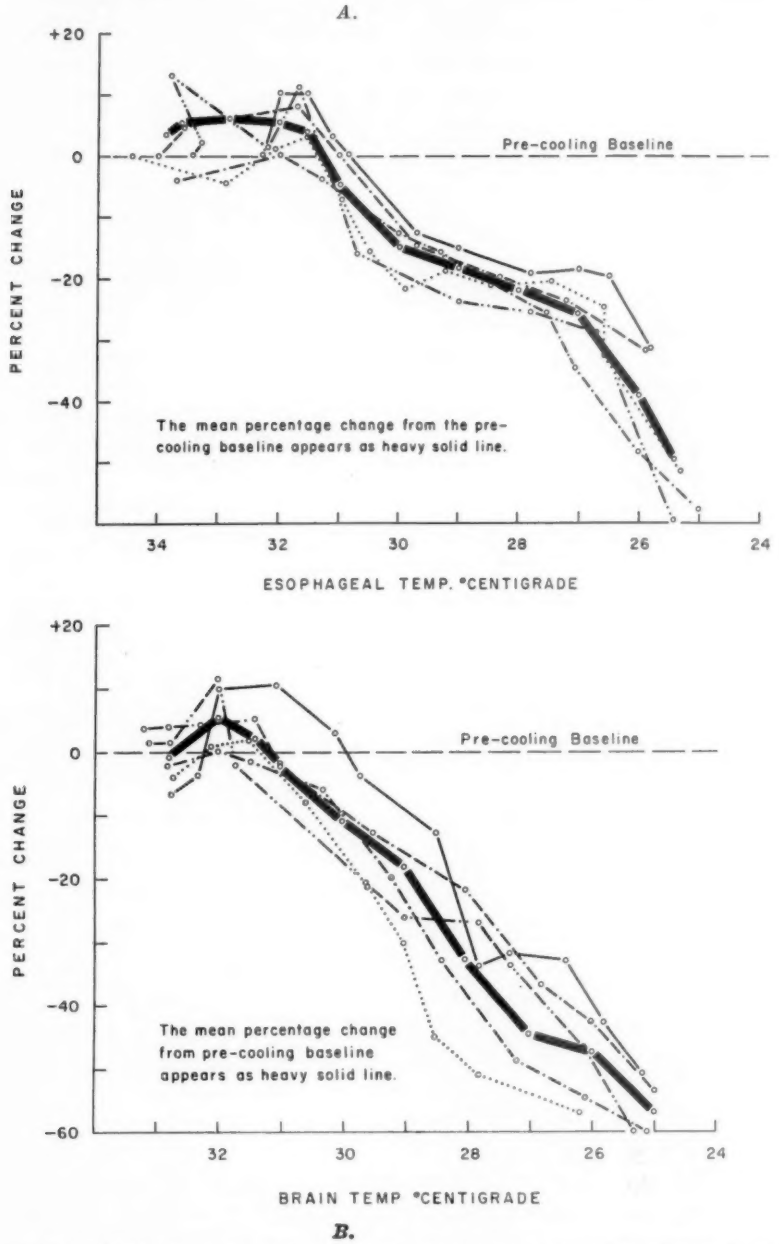

Fig. 2.—The range of carotid blood flow change with change in *(A)* esophageal and *(B)* brain temperature in cats.

tion usually caused an abrupt fall in carotid blood flow in the head-erect posture. Because of this, our most reliable data for carotid flow were obtained with the animal respiring spontaneously. The effects of administration of curare upon blood pressure and carotid flow are illustrated in Fig. 5. With a reduction in

temperature, an erratic but definite fall in carotid flow was observed following an initial rise immediately after immersion in the ice brine. The chart of flow in the common carotid arteries as a function of brain temperature is given

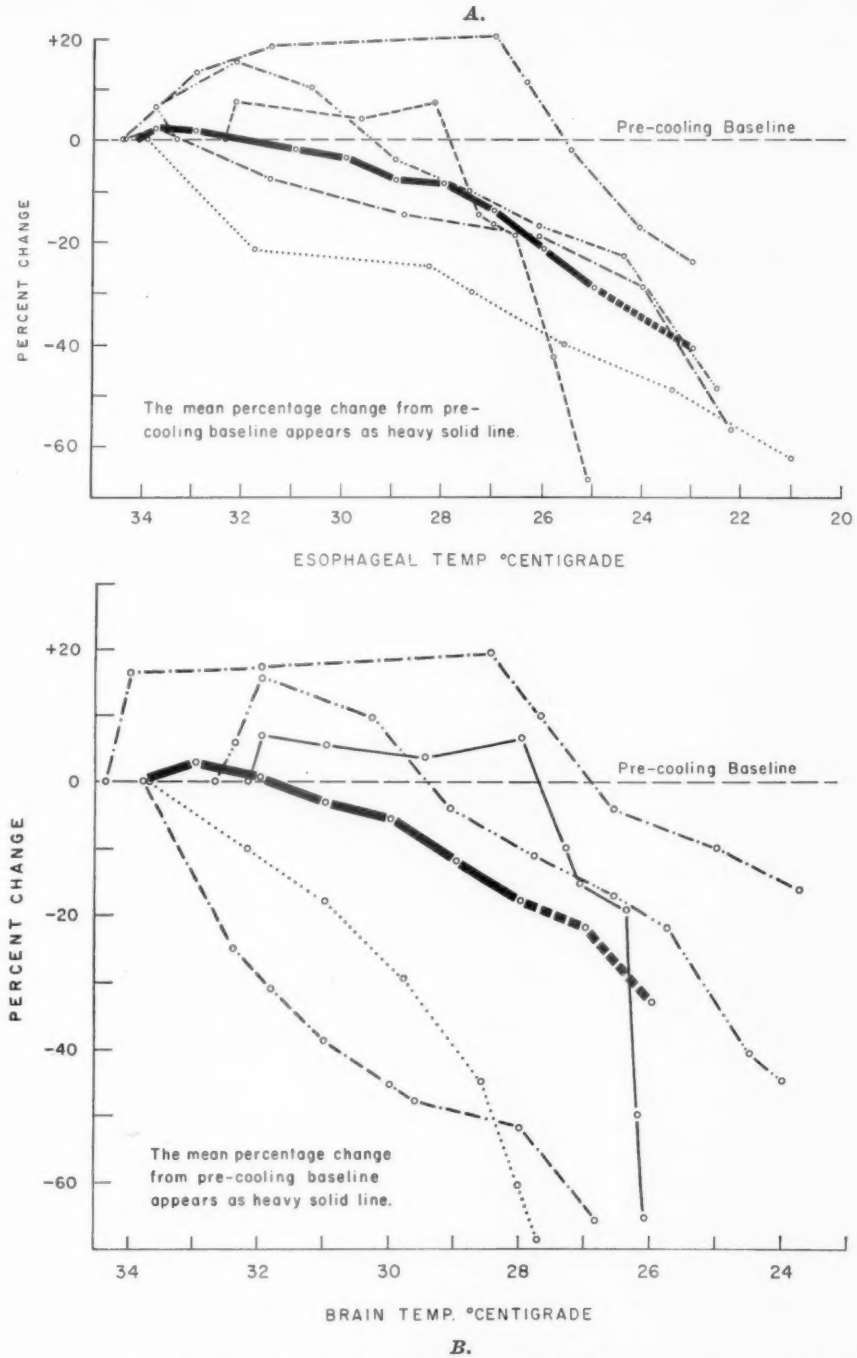

Fig. 3.—The range of cisternal oxygen tension change with change in (*A*) esophageal and (*B*) brain temperature in cats.

in Fig. 6. In the 4 animals it can be seen that the percentage fall below the precooling base line was 40 per cent or more at brain temperatures between 32.5°

and 29° C. As the monkeys were returned to a warm bath (40° C.), a rise in blood flow was recorded which bore no relationship to body temperature. In at least 2 animals the recorded temperatures fell further while carotid flow increased. No significant rise in femoral arterial blood pressure was recorded along with these rises in carotid blood flow.

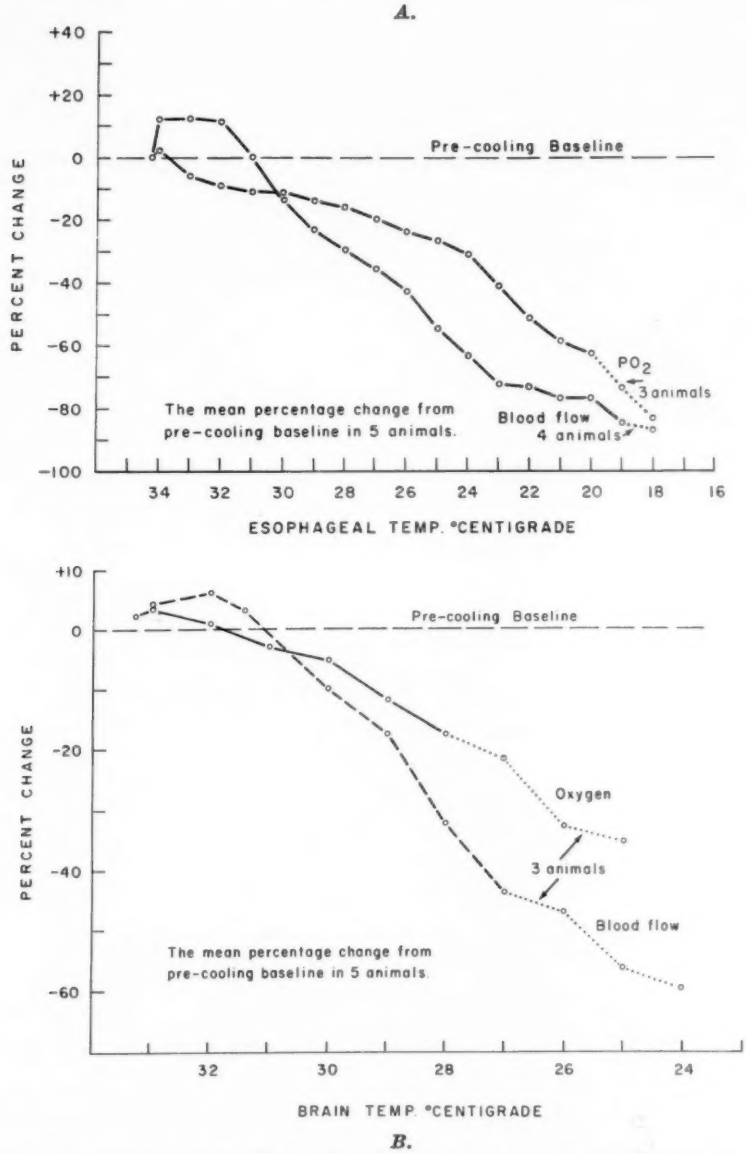

Fig. 4.—The mean percentage of carotid blood flow and cisternal oxygen tension plotted against (*A*) esophageal and (*B*) brain temperature in cats.

Cisternal oxygen tension: The mean precooling value in 5 animals was 62 ± 15 mm. Hg oxygen tension. Reduction in temperature was associated with a reduction in cisternal oxygen tension in 4 out of 5 animals. The graphs of the percentage fall from the precooling base line for both esophageal and brain temperature are presented in Fig. 7, *A* and *B*. At brain or esophageal temperatures of 30° C., the mean percentage fall in tension from the base line was

18 to 19 per cent. In each of 3 monkeys in which rewarming was accomplished, the oxygen tension rose prior to a reversal of the animal's temperature curve and reached the base line at temperatures which, during the initial cooling phase, were associated with lower tension values.

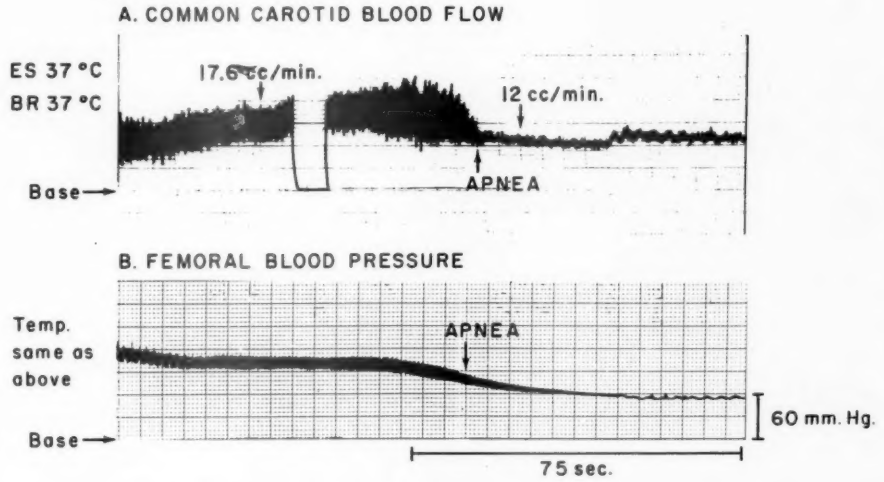

Fig. 5.—The normothermic response to the intravenous administration of curare to the point of respiratory paralysis in the head-erect posture in the monkey. The figures for blood flow indicate mean arterial flow.

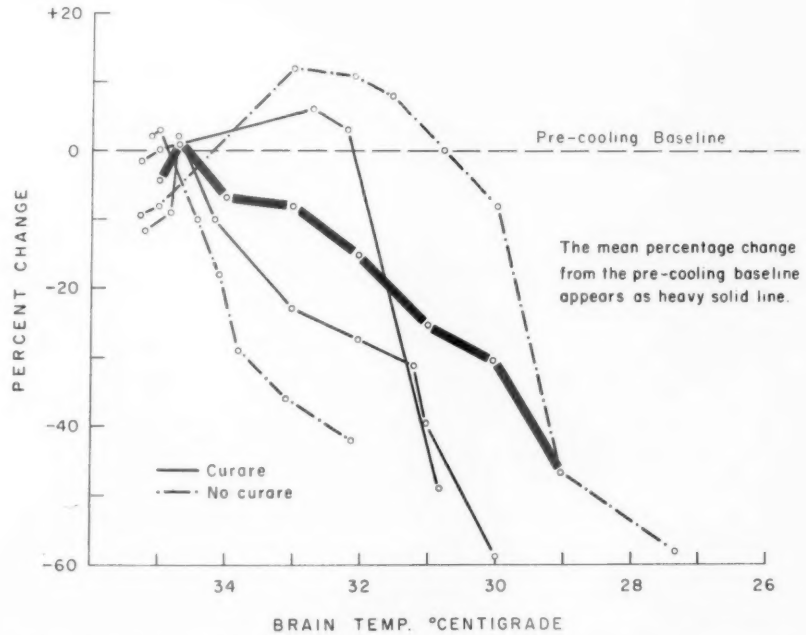

Fig. 6.—The range of carotid blood flow change with change in brain temperature in monkeys (4 animals).

The relationships between oxygen tension, carotid blood flow, and temperature are presented in Fig. 8, *A* and *B*, for both brain and esophageal temperatures. The cisternal oxygen tension fell less abruptly than did carotid flow. A reduction in bilateral carotid flow exceeded, in per cent change, the oxygen tension decline below 32° C. brain temperature or 33° C. esophageal temperature.

With a fall in temperature, the response, either of blood pressure or cisternal oxygen tension or both, to ischemic anoxia or anoxic anoxia was reduced. The normothermic response to ischemic anoxia was a transient rise in systemic blood pressure. A slight but probably not significant oxygen tension

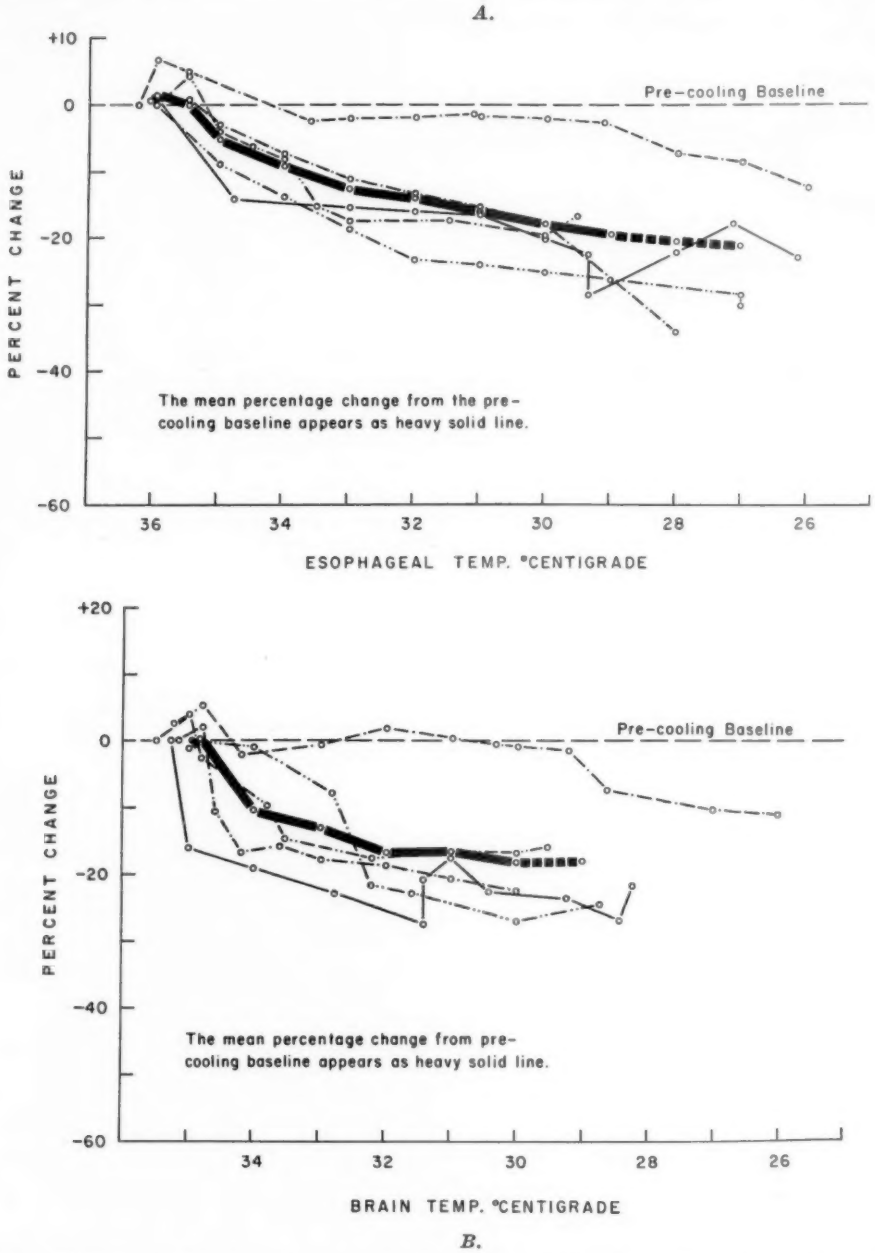

Fig. 7.—The range of cisternal oxygen tension change with change in (*A*) esophageal and (*B*) brain temperature in monkeys (5 animals).

fall occurred. At normothermic levels anoxic anoxia produced a sharp rise in blood pressure, a fall in oxygen tension, and a secondary delayed blood pressure rise following the resumption of normal respiration (Fig. 9). Ischemic anoxia produced by bilateral carotid occlusion at temperatures of 27° C. in the

esophagus or 29.6° C. in the brain produced no blood pressure or oxygen tension change (Fig. 10). Below temperatures of 30° C. in the esophagus or 32.2° C. in the brain, the blood pressure response to anoxic anoxia was markedly reduced, and no change was measured in the oxygen tension. Rewarming of the

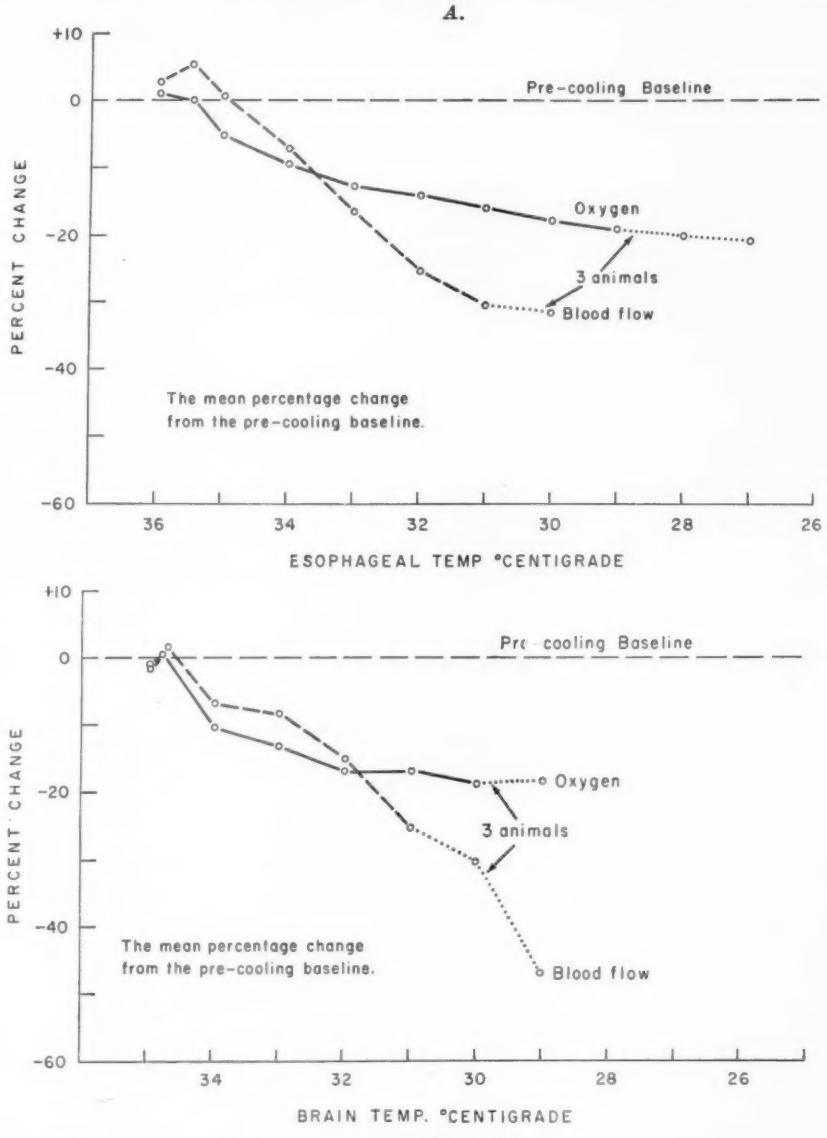

Fig. 8.—The mean percentage change of carotid blood flow and cisternal oxygen tension plotted against (*A*) esophageal and (*B*) brain temperature in 5 monkeys.

animal was associated with a return of the blood pressure response to ischemic and anoxic anoxia but with a less constant change in oxygen tension, namely, a mild rise with ischemic anoxia and an initial rise followed by a fall with anoxic anoxia (Fig. 11).

REVIEW OF RELATED STUDIES

Determinations of arteriovenous oxygen differences have been used to reflect oxygen utilization by the brain. In 1939 Fazekas and Himwich[5] studied the

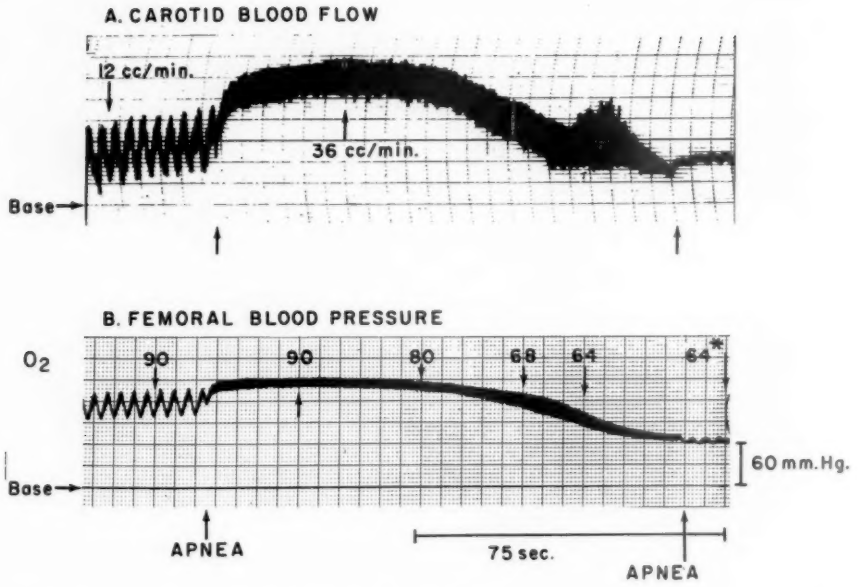

Fig. 9.—The normothermic response to respiratory arrest in the monkey. The period of apnea is indicated between the vertical arrows. Note the fall in cisternal cerebrospinal fluid oxygen tension indicated on the femoral pressure tracing. Resumption of respiration was followed by an overshoot of blood pressure and a return of oxygen tension to 97 mm. Hg.

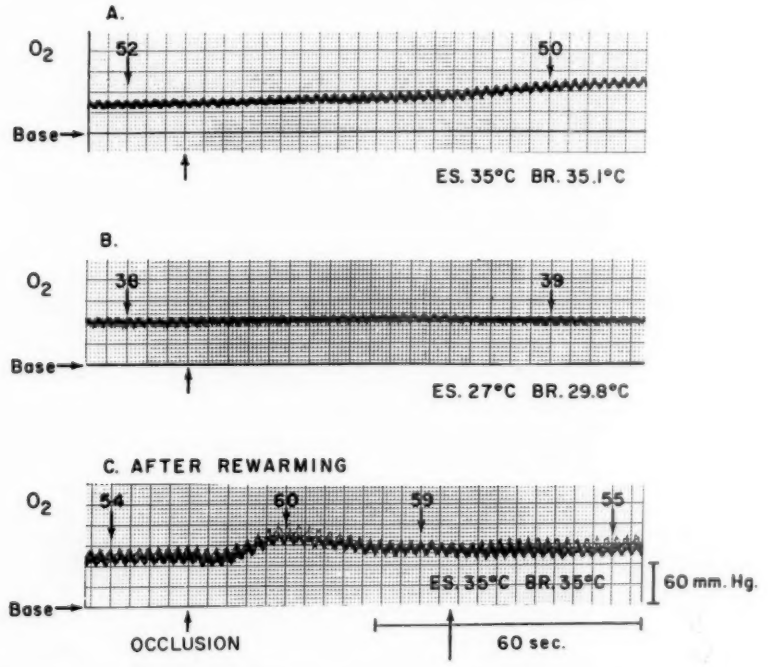

Fig. 10.—Responses of cisternal oxygen tension and femoral blood pressure to 60 seconds of bilateral carotid occlusion at normo- and hypothermic levels in the monkey. Note the reduced response at the lowered temperature.

effects of external body cooling by reducing the rectal temperature of dogs to 26° C. The cerebral arteriovenous oxygen differences fell as the rectal temperature fell. These authors found that the superior longitudinal sinus venous blood oxygen rose, while the arterial oxygen remained relatively stable. The heart rate fell and circulation time was diminished (in themselves these should produce an increased arteriovenous difference if the brain metabolism of oxygen were stable). These workers reasoned that the reduced arteriovenous differences pointed to a diminution of cerebral metabolism as a result of the cooling. A reduction of cerebral arteriovenous oxygen difference to 1.52 to 2.0 volumes per

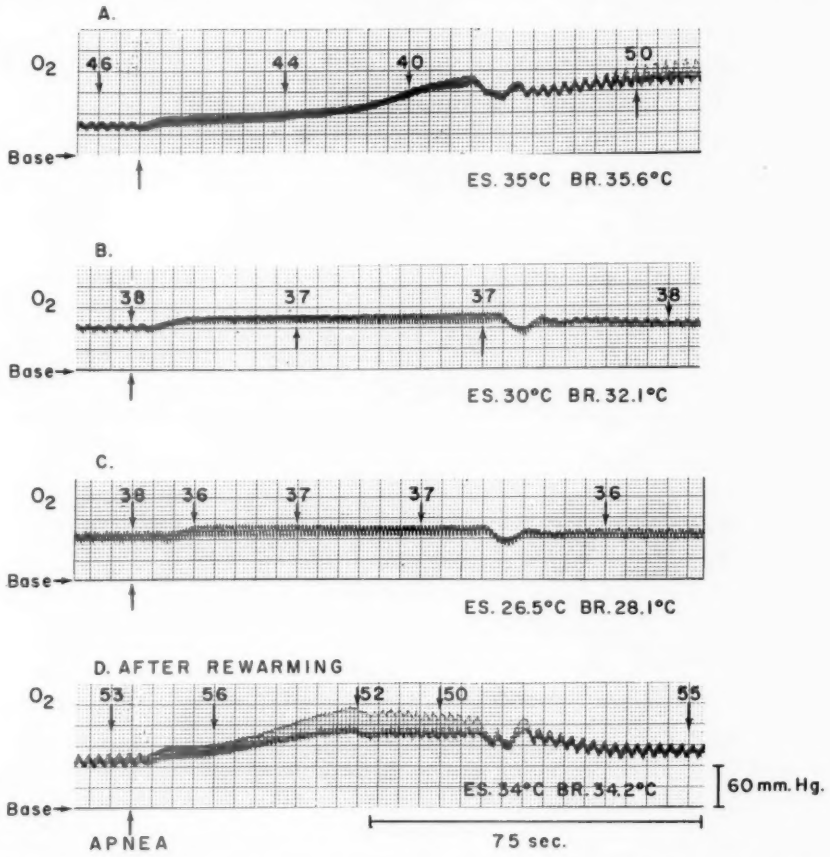

Fig. 11.—Normothermic and hypothermic responses of systemic arterial pressure and cisternal cerebrospinal fluid oxygen tension to 75 seconds of respiratory arrest in the monkey. Note the reduced responses at lowered temperatures.

cent at 20° C. was reported in dogs and cats by Libet and associates.[10] Field, Fuhrman, and Martin[6] demonstrated the effects of temperature on the oxygen uptake of rat cerebral cortex slices. They found that the QO_2 is a continuous function of temperature and that in the ranges of 10° to 37.5° C. the log QO_2 as a function of C.° temperature is linear.

Lougheed and Kahn,[11] in 1955, utilized experimental systems with controlled cerebral blood flow and regional cooling as well as systemic total body cooling without control of flow. In each system, the arteriovenous oxygen differences fell and, as expected, were more strikingly reduced in the dogs with controlled blood flow (regional brain perfusion). The cerebral metabolic rate for oxygen

was calculated by these authors to be from 23 to 35 per cent of normal at 25° C. They found no evidence of anoxia having been produced by the hypothermia, and concluded that the oxygen consumption was reduced rather than its supply being restricted by an inability of the brain to extract oxygen at the lower temperature.

Bering and colleagues[2] found in monkeys that with the use of artificial respiration and the avoidance of shivering, cerebral oxygen consumption (calculated on the basis of cerebral arteriovenous oxygen differences and blood flow by the Kety-Schmidt method) did not alter much down to 31° C. Below this temperature, and down to 27° C., a sudden decrease in consumption occurred. They calculated that at 26° C. oxygen consumption by the brain was 25 to 30 per cent of normothermic levels. The cerebral arteriovenous oxygen difference was inversely correlated with cerebral blood flow.

Rosomoff and Holaday,[16] utilizing dogs artificially respired, measured blood flow by means of a magnetic rotometer. They calculated cerebral oxygen consumption as a product of cerebral blood flow and the arteriovenous oxygen difference. They expressed cerebral oxygen consumption, not as cubic centimeters of oxygen extracted by the brain per 100 c.c. of blood,[11] but rather as cubic centimeters of oxygen per 100 grams of brain per minute. With external body cooling, they found a fall in cerebral blood flow of 6.7 per cent per degree centigrade, but a stable arteriovenous oxygen difference at temperatures down to 26° C. By their method of calculation, therefore, oxygen consumption and cerebral blood flow varied in the same direction and to the same degree with the decrease in temperature. At 26° C. the oxygen consumption (cerebral metabolic rate of Lougheed and Kahn[11]) was 34 per cent of normothermic levels.

In 1956, Stone, Donnelly, and Frobese[17] carried their observation to man. Controlled respiration was not used and shivering was controlled in only 2 of 3 patients. In these 2 patients cerebral arteriovenous oxygen differences fell with Pentothal anesthesia and rose again during hypothermia but not to control levels. The cerebral blood flow (Kety-Schmidt method) remained unchanged with Pentothal but fell sharply at 83° F. and 84.8° F., respectively. On the basis of the fall of flow, there was calculated a marked reduction in cerebral metabolic rate or oxygen consumption expressed in cubic centimeters of oxygen per 100 grams brain weight per minute. Flow fell 72 per cent and oxygen consumption 76 per cent at 83° F. and flow fell 52 per cent and oxygen consumption 61 per cent at 84.8° F. A respiratory acidosis existed with each case. If shivering occurred (their third case), cerebral blood flow fell less (12 per cent) and oxygen consumption increased (105 per cent).

At temperatures of 29° C., Adams and associates[1] found in 8 patients a reduction in cerebral blood flow (Kety-Schmidt method), and a reduction of cerebral metabolic rate for oxygen. The latter reduction was approximately 50 per cent of normothermic levels.

It should be evident that the workers up to this point utilized blood flow and blood gaseous measurement to estimate oxygen consumption and, indirectly, cerebral oxygen availability. In 1946 Bronk[3] reviewed his experience with the

method of Davies and Brink of measuring the reduction of dissolved oxygen expressed as current flow between the poles of an electrode placed directly on brain tissue. It was evident that to measure oxygen consumption, supply must be constant or at least controlled. The brain's consumption of oxygen kept the concentration low, thereby favoring a rapid transfer of oxygen from the blood.

Meyer, Fang, and Denny-Brown,[13] utilizing a similar polarographic principle in cats and monkeys, confirmed the presence of a critical systemic blood pressure of about 60 mm. Hg and a short oxygen reserve time (10 seconds) which, if exceeded, resulted in a fall in oxygen availability as measured by the polarograph in the brain.

Meyer and Denny-Brown[12] reported that the particular electrode of their design measured oxygen availability in brain tissue. This availability would be dependent upon (1) local blood flow, (2) oxygen concentration in the general circulation, and (3) local metabolic consumption. In 1957 Meyer and Hunter[14] measured cortical tissue oxygen availability by the polarographic technique in both animal and man. They concluded that neuronal metabolism was reduced by cold, but they failed to find a significant reduction of tissue oxygen available to the cell or in carotid artery blood with the reduction of temperature from 37° to 21° C.

Kaplan and colleagues,[7] utilizing chronically implanted electrodes (Clark[4]) in dogs, studied the azygos flow principles, total inflow occlusion, and the breathing of various oxygen concentrations. They reported the cathode output to be a function of oxygen available in the immediate environment and capable of measuring oxygen brought by diffusion and mechanical fluid transport. They suggested that a reduction of cerebral blood flow may conceivably result in an abrupt decrease in oxygen availability while tissue oxygen tension falls at a slower rate. These authors believed it is possible that the protection afforded the brain by hypothermia may not depend only on a decreased oxygen need.

DISCUSSION

Important variables in studies of this type require attention.

Respiratory Control.—Hypothermia produces a slowing of rate and eventual irregularities. The venous pressure may thereby be increased and circulation time increased. A respiratory acidosis often occurs. Artificially controlled respiration requires the use of pharmacologic agents which may influence blood flow as a function of pressure and neuronal metabolism.

Shivering.—Hypothermia induces shivering which, if uncontrolled, increases oxygen requirements of tissue. Although the brain appears to participate in this increased metabolism with shivering, the reason for this is not clear.

Blood Flow Measurement.—The gaseous exchange technique of Kety and Schmidt does not permit a continuous recording of flow and may not therefore sample important transient changes in flow. The perfusion flow methods require the cutting of the artery and interruption of perivascular neural plexuses.

Species and Genus Variations.—Rats, cats, dogs, monkeys, and man have supplied data to the various investigators whose work is reviewed. The sharp

anatomic and presumed functional differences between canine and human cerebral circulation are recognized. The monkey is a compromise, more closely approximating man's pattern than do other animals.

The values of oxygen tension (mm. Hg) would seem to depend upon the oxygen concentration in the general circulation, the local blood flow in the environment of the electrode, and the local utilization of oxygen. The cerebrospinal fluid is looked upon as part of the extracellular space of the brain, and oxygen readily diffuses throughout this fluid. We must make a basic assumption that cerebrospinal fluid oxygen tension reflects the equilibrium or the gradient between supply to and utilization by the brain tissue of oxygen. Having made this assumption and having assured an adequate supply of oxygen to the blood via the respiratory system, we may then reason as to the significance of a fall of cisternal oxygen tension with hypothermia. This finding suggests that, as temperature falls, less oxygen is released from the blood to diffuse through the tissue fluids and be available to the cells. If this oxygen availability is less than needed, tissue anoxia would supervene promptly and signs of anoxia would be expected in alterations in blood pressure and blood flow. Both of these latter functions were seen to fall and, in terms of per cent, to a greater degree than oxygen availability. This information supports the concept that rather than the production of anoxia by less than optimal availability of oxygen, there is a decreased utilization by virtue of decreased need, i.e., a decreased metabolism with lowered temperature. The observations upon the blood pressure and oxygen tension responses to ischemia and anoxic anoxia at lowered temperatures further suggest that either the carotid reflexes are less sensitive or there is no hypoxia stimulus. The oxygen available to the tissue seems to be greater in proportion than is blood volume at lower temperatures. If blood flow were sharply reduced and oxygen tension (or availability) preserved at a relatively higher level, this would point to either (1) a greater uptake of oxygen by the tissue from the blood, and presumably then a greater arteriovenous oxygen difference would be recorded, or (2) a decreased utilization by the tissue in the face of adequate oxygen diffusion. The majority of workers have reported a decreased cerebral arteriovenous oxygen difference rather than a rise. This information, coupled with our data, supports the concept of decreased utilization of oxygen by the tissue during hypothermia.

The carotid blood flow data utilized in these discussions represent mean common carotid flow. No attempt was made to isolate the intracranial supply from the remainder of the flow, and hence the absolute figures given for flow cannot be considered the same as cerebral blood flow. Our figures are expectedly high when compared with dog rotometer data of Rosomoff and Holaday[16] or the Kety-Schmidt data of the monkeys of Bering and associates.[2] That the brain will share in the flow changes proportionate to the variation in the data we have seems a reasonable assumption.

From these studies it would appear that for surgical procedures designed to interfere with cerebral circulation, whether in the thorax, neck, or cranium, the optimum temperature of the brain should be achieved which produces the greatest divergence in the degree of fall in carotid flow and oxygen availability.

The temperature at which this occurs varies from one animal to another and has a range which seems to be between 32° and 26° C. The proportionately greater fall in blood flow compared with oxygen tension began at 29° C. in the cats and between 31° and 32° C. in the monkeys. Bering and his co-workers[2] suggested the range 31° to 27° C. At such lowered temperatures, there is a reduction of blood pressure and oxygen tension responses to anoxia. Although the carotid sinus reflexes and chemoceptor reflexes have been shown to be depressed in the hypothermic cat,[15] the lack of appreciable change in the oxygen tension during our maneuvers to produce transient anoxia suggests that this depression, which we also observed, represented a decreased susceptibility of the receptor system to the ischemia or anoxia by virtue of lack of tissue need rather than potentially harmful reduction of reflex activity per se with a resulting threat of tissue anoxia.

On the basis of the cited reports of reduction in oxygen consumption at lowered body temperatures, it would appear that with hypothermia there is a greater oxygen availability than there is consumption and that the greatest brain protection exists when this difference is maximum. The limiting factor of increased cardiac irritability in the mid-20° C. temperature range is well recognized.

The utilization of curare-like preparations to produce respiratory arrest renders the animal particularly susceptible to a fall in blood flow in the carotid system when the head is placed in an erect posture. Such a factor may be of considerable importance when the sitting or erect posture is utilized in intracranial or cervical surgery, particularly if combined with hypothermia.

The information on rewarming of 3 monkeys suggests that even though the body and brain temperatures remain low or even fall further, there is a rise in carotid blood flow and cisternal oxygen tension without a corresponding rise in mean systemic arterial blood pressure. These data suggest a decrease in carotid arterial tree resistance favoring more efficient oxygenation. Whereas no shocklike condition was observed, it might be worth comment that if carotid system vascular resistance can be altered in this fashion, other vascular beds might also be so altered as to permit the animal to pool large volumes of blood and produce adverse effects.

SUMMARY AND CONCLUSIONS

An experimental study of the effects of total body cooling has been undertaken in cats and monkeys with the use of the polarographic technique of determining cisternal cerebrospinal fluid oxygen availability and the electromagnetic blood flow principle to determine carotid blood flow.

Although brain temperatures lagged behind changes in esophageal temperature, the two followed the same curve.

Carotid blood flow fell disproportionately more than did systemic arterial blood pressure as body temperature fell. This indicated an increased vascular resistance in the carotid arterial system.

Cisternal oxygen availability was assumed to reflect oxygen diffusing from the arterial system into the fluid environment of cerebral tissue. This oxygen

availability was found to fall as the animal was cooled, but the percentage of fall was less than the fall in carotid arterial flow below temperatures of 31° C. (brain) in the monkeys and 29° C. in the cats.

At temperatures below the range of 31° to 29° C., the disproportionate fall in flow compared with oxygen availability strongly suggests a decreased utilization of oxygen by the tissues and therefore increased brain protection against anything which might produce anoxia.

The normothermic responses of blood pressure and cisternal oxygen tension to ischemic and anoxic anoxia were sharply reduced at hypothermic levels, suggesting at least a decreased sensitivity to and probably increased tolerance to anoxia.

The techniques employed in this study have given data which support the conclusions of most other workers, that the brain is maximally protected against anoxia at body temperatures below 31° C. Consideration is given to the effects of the head-erect posture, the utilization of curare-like agents, and the application of rewarming techniques, each of which is associated with alterations in oxygen tension, carotid blood flow, or blood pressure.

REFERENCES

1. Adams, J. E., Elliot, H., Sutherland, V. C., Wylie, E. J., and Dunbar, R. D.: Cerebral Metabolic Studies of Hypothermia in the Human, S. Forum **7**: 535-539, 1957.
2. Bering, E. A., Jr., Taren, J. A., McMurrey, J. D., and Bernhard, W. F.: Studies on Hypothermia in Monkeys—II. The Effect of Hypothermia on the General Physiology and Cerebral Metabolism of Monkeys in the Hypothermic State, Surg. Gynec. & Obst. **102**: 134-138, 1956.
3. Bronk, D.: The Physical Structure and Biological Action of Nerve Cells, With Some References to the Problems of Human Flight, Am. Scientist **34**: 55-76, 1946.
4. Clark, L. C., Jr., Wolf, R., Granger, D., and Taylor, Z.: Continuous Recordings of Blood Oxygen Tensions by Polarography, J. Appl. Physiol. **6**: 189-193, 1953.
5. Fazekas, J. F., and Himwich, H. E.: Effect of Hypothermia on Cerebral Metabolism, Proc. Soc. Exper. Biol. & Med. **42**: 537-538, 1939.
6. Field, J., Fuhrman, F. A., and Martin, A. W.: Effect of Temperature on the Oxygen Consumption of Brain Tissue, J. Neurophysiol. **7**: 117-126, 1944.
7. Kaplan, S., Matthews, E. C., Schwab, L., and Clark, J. C., Jr.: Oxygen Availability to the Brain During Inflow Occlusion of the Heart in Normothermia and Hypothermia, J. Thoracic Surg. **32**: 576-582, 1956.
8. Kolin, A.: An Electromagnetic Flowmeter. Principle of the Method and Its Application to Bloodflow Measurements, Proc. Soc. Exper. Biol. & Med. **35**: 53-56, 1936.
9. Kolin, A.: Electromagnetic Blood Flow Meters, Science **130**: 1088-1097, 1959.
10. Libet, B., Fazekas, J. R., Meirowsky, A. M., Campbell, E. H., and Himwich, H. E.: Control of Electrical and Oxidative Activity of Brain by Temperature, Am. J. Physiol. **129**: 404-405, 1940.
11. Lougheed, W. M., and Kahn, D. S.: Circumvention of Anoxia During Arrest of Cerebral Circulation for Intracranial Surgery, J. Neurosurg. **12**: 226-239, 1955.
12. Meyer, J. S., and Denny-Brown, D.: Studies of Cerebral Circulation in Brain Injury, I. Validity of Combined Local Cerebral Electropolarography Thermometry and Steady Potentials As an Indicator of Local Circulatory and Functional Changes, Electroencephalog. & Clin. Neurophysiol. **7**: 511-528, 1955.
13. Meyer, J. S., Fang, H. C., and Denny-Brown, D.: Polarographic Study of Cerebral Collateral Circulation, A. M. A. Arch. Neurol. & Psychiat. **72**: 296-312, 1954.
14. Meyer, J. S., and Hunter, J.: Effects of Hypothermia on Local Blood Flow and Metabolism During Cerebral Ischemia and Hypoxia, J. Neurosurg. **14**: 210-227, 1957.
15. Nashat, F. S., and Neil, E.: The Effect of Hypothermia on Baroceptor and Chemoceptor Reflexes, J. Physiol. **127**: 59-60P, 1955.
16. Rosomoff, H. L., and Holaday, D. A.: Cerebral Blood Flow and Cerebral Oxygen Consumption During Hypothermia, Am. J. Physiol. **179**: 85-88, 1954.

17. Stone, H. H., Donnelly, C., and Frobese, A. S.: The Effect of Lowered Body Temperature on the Cerebral Hemodynamics and Metabolism of Man, Surg. Gynec. & Obst. **103**: 313-317, 1956.
18. Wetterer, E.: Eine neue Methode zur Registrierung der Blutströmungsgeschwindikeit am uneröffneten Gefäss, Ztschr. f. Biol. **98**: 26-36, 1937.

Discussion

DR. JOHN E. ADAMS (San Francisco, Calif.).—I think Dr. Stern is to be congratulated for this very logical and direct approach to the study of the problem as to how the brain is protected by hypothermia. Previous work on this problem has largely been directed toward studying the effect of hypothermia on cerebral blood flow, and oxygen metabolism as measured by arteriovenous oxygen differences. It would appear that measurement of oxygen tension is the more crucial parameter. However, I am concerned about his using the oxygen tension of the cerebrospinal fluid in the basal cistern as a measurement even indirectly of the oxygen tension of the brain. The oxygen tension of jugular bulb venous blood probably closely approximates that of the brain. If we increase arterial oxygen tension above normal levels the tension of the jugular bulb, and I think that of the brain, increases slightly if at all. This has been the case in a few instances where we have followed the oxygen tension of the brain utilizing the same microelectrode. If the change in arterial oxygen tension is in the reverse direction, perhaps spinal fluid oxygen tension is representative of tissue oxygen tension.

I am wondering, therefore, if when Dr. Stern measures the oxygen tension of the cerebrospinal fluid of the basal cistern he is not actually measuring the changes in oxygen tension as a result of diffusion of oxygen from the choroid plexus rather than the brain or at least a combination of the two. In spite of this possible objection to his data, I commend him for the application of this method in this most interesting study.

DR. SAMUEL R. POWERS (Albany, N. Y.).—I would like to make a comment about the techniques about the use of the oxygen tension devices. I would like to ask how these devices are calibrated with respect to temperature because as you well know the oxygen tension electrodes depend on a physical chemical reaction which is in itself extremely temperature-sensitive so that if you take a blood sample and place it in a closed space where no change in quantity of dissolved oxygen could occur and then vary the temperature, of course, the oxygen tension will read entirely differently so that I wonder whether the oxygen tension measurements, which were reported at varying temperatures, were a reflection of changes in oxygen concentration or changes in physical chemical reactions. The second thing I would like to mention is that also the availability, of course, depends upon the gradient of oxygen tension between the blood and the cell and, since presumably the tissue and the blood are having their temperature lowered simultaneously, one might find a lowered oxygen tension and yet actually the gradient across the cell membrane might remain exactly the same so that availability would be unchanged.

DR. WILLIAM E. NEVILLE (Cleveland, Ohio).—About a year ago, George Clowes and I, following the lead of the group at Duke, interposed one of Ivan Brown's heat exchangers in the arterial line from our membrane oxygenator. In conjunction with our neurosurgeon, Barney Bloor, we measured the oxygen tension with an electrode developed by Dr. Bloor in the cistern and the sagittal sinus, in dogs on total perfusion and cooled to 10° C.

As you see in this first slide, as the temperature of the brain falls, the arterial oxygen tension rises quite markedly. You will notice that the percentage saturation of the carotid artery remains the same at normothermic and at hypothermic levels. Concomitantly, the percentage saturation of the sagittal sinus rises quite markedly with a resultant arteriovenous difference which at 10° C. is nil. Also, you will notice that the pO_2 of the sagittal sinus falls with the drop in brain temperature.

This second slide depicts high, medium, and low flows at 37° and 15° C. The oxygen content of the arterial blood remains the same. There is a marked A-V difference in the high, medium, and low flow rates at 37° C. and practically no A-V difference at 15° C.

If you will notice, though, the sagittal sinus pO_2 is lower at 15° than at 37° C. Even though the venous blood in the sinus is perfectly red at 15° C. the pressure of oxygen is lower than at normothermic levels. This means that we may be fooling ourselves at this severe degree of hypothermia into thinking that since there is a negligible A-V difference, we are adequately satisfying the oxygen requirements of the patient's brain.

This last slide depicts the typical temperature gradient present in animals and patients whose esophageal and brain temperatures have been lowered to 10° C. This patient had his entire cerebral circulation shut off for 30 minutes. His total body circulation was completely interrupted for an additional 12 minutes in order to extirpate a hemangioma of the brain. You will notice that the temperature of the brain is 10° C. and the esophageal temperature is about 7° C. However, the rectal temperature is 20° C. This means that even though there is a negligible A-V difference at 10° C., the body is still utilizing oxygen because of this discrepancy in temperature between the big muscle mass in the buttocks and the brain and esophagus.

DR. STERN (closing).—I would like to thank Dr. Adams, Dr. Powers, and Dr. Neville for their comments. To Dr. Adams—we, too, are concerned about the fact that the cisternal cerebrospinal fluid may not have a very close relationship to carotid arterial supply. The relative size of choroid plexus to surface area of the great cistern is much smaller than in the case of the lateral ventricle and therefore the objection that our data may represent oxygen diffusion from the choroid plexus per se may not be quite as important. I think, however, that this is diffused oxygen which is being recorded in the cerebrospinal fluid, and it is oxygen from the blood reaching the intracranial chamber. We reason that it is also, therefore, available to the brain. Kaplan and others have implanted electrodes of this general type chronically and have measured oxygen tensions within the brain tissue itself. The technique is available, but we have not mastered it at the present time. Dr. Powers' cogent comments obviously are pertinent to this discussion. We calibrated the electrode for temperature utilizing a known oxygen concentration in a solution, the temperature of which could be varied, and corrected our readings from the analyzer accordingly. With respect to availability, I think Dr. Neville, in part, answered this question by presenting some of the very nice work which Dr. Bloor has been doing in Cleveland. Meyer and Hunter's work also demonstrated that, during hypothermia, there was no fall in the oxygen content of the arterial blood and, therefore, this provided an adequate oxygen supply. The oxygen gradient was such as to enhance the brain oxygen supply.

MYOCARDIAL METABOLISM AND POSTARREST FUNCTION IN THE COLD AND CHEMICALLY ARRESTED HEART

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THE repair of many cardiac defects by extracorporeal techniques requires a bloodless and motionless field. Several methods have been used to produce elective cardioplegia, but no one method has gained universal acceptance. Potassium citrate,[18] acetylcholine,[16] and a mixture of magnesium, neostigmine, and potassium[24] are agents which have been used extensively. However, dissatisfaction with these chemical cardioplegic agents has prompted some surgeons to resort to simple occlusion of the ascending aorta to produce a relatively bloodless field. This ischemic method produces cardiac arrest in 8 to 15 minutes, but irreversible myocardial damage may occur after 15 to 20 minutes and limits this technique.[7, 11] Recently, differential hypothermic cardioplegia has been introduced and successfully used in human beings.[26]

In order to assess properly each method of producing cardioplegia, the postarrest ventricular function and the cellular metabolism and microscopic anatomy of the arrested heart must be studied. Clinical impressions are not subject to quantitative comparison, and although these impressions are important, they may be misleading.

Helmsworth and associates[13] and Reynolds and his colleagues[22] have demonstrated microscopic structural changes in canine hearts arrested 30 minutes with potassium citrate. Furthermore, Willman and his group[31] have demonstrated a pronounced depression in postarrest left ventricular function after 20 and 30 minutes of potassium arrest. To date no comparable studies of other cardioplegic methods have been published.

In order to evaluate critically current cardioplegic methods, we produced a standard 1-hour cardiac arrest in a series of 47 dogs with potassium citrate, acetylcholine, or cold. The series was divided into 2 groups. In the first group oxygen utilization and lactic acid metabolism were studied before, during, and after each arrest. The chemical cardioplegic methods were investigated at both 37° and 28° C. In the second group left ventricular work capacity was determined before and after cardiac arrest, while myocardial oxygen availability was monitored during the arrest. Correlative histologic examinations completed the evaluation.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

EXPERIMENTAL METHOD

Healthy mongrel dogs weighing 13 to 25 kilograms were anesthetized with intravenous thiopental and ventilated mechanically with 100 per cent oxygen. The electrocardiogram was monitored during the experiment. Arterial and venous pressures were recorded by means of indwelling catheters connected to a Statham strain gauge or a saline manometer. After a midline sternal thoracotomy was performed, heparin (1 mg. per kilogram) was given intravenously. Blood, esophageal, and heart temperatures were measured with thermistor probes. Appropriate catheters placed in the innominate and femoral veins were led to a Kay-Cross oxygenator and a piston pump perfusion system. Blood was returned to the animal through a femoral arterial catheter. Thermoregulation of the blood was accomplished by means of a heat exchanger placed inside the oxygenator cylinder.[27] After ligation of the azygos vein, venous inflow occlusion was accomplished by encircling caval tourniquets. Pump flows of 40 to 70 c.c. per kilogram per minute maintained satisfactory perfusion at 70 to 120 mm. Hg arterial pressure.

Metabolic Group.—In 27 animals a separate extracorporeal system consisting of a small Kay-Cross oxygenator and manual pump was used to perfuse the isolated heart. A stiff arterial catheter from this pump was placed at the root of the aorta through the brachiocephalic artery. A tourniquet was passed around the ascending aorta so that the coronary perfusion system could be completely isolated from the systemic circulation. A multiperforated catheter was introduced into the right ventricle and also into the right atrium to collect all coronary venous return. Blood from this catheter was returned to the extracorporeal coronary oxygenator via a graduated cylinder flowmeter. A tourniquet around the pulmonary artery and a left atrial vent insured isolation of the coronary from the systemic circulation.

After prearrest control samples were obtained, the cardioplegic drug or cold blood diluted with dextran was perfused through the isolated coronary system. A heat exchanger within the oxygenator was used to regulate myocardial temperature during the arrest. Potassium arrest was easily maintained with a perfusate concentration of approximately 15 mEq. potassium citrate per liter. However, after 200 mg. of acetylcholine had been given initially, over 1 Gm. of additional acetylcholine was required to sustain the arrest for 1 hour. During hypothermic asystole cold saline was applied externally to the surface of the heart to keep the cardiac temperature below 10° C.

In 12 animals with hearts arrested by cold, oxygen consumption studies were obtained at various temperatures during cooling and rewarming. The heart temperature was stabilized for 3 minutes after which the appropriate samples were obtained every 10 or 15 minutes during the hour of arrest. Following arrest, the aortic tourniquet was released to allow perfusion of the heart by the systemic extracorporeal circulation. Metabolic studies were continued during the immediate postarrest period. When necessary, defibrillation was accomplished by electric shock.

Oxygen content of the blood was determined by the manometric method of Van Slyke and Neill.[29] The colorimetric method of Barker and Summerson[2] was used for the lactic acid studies. Serum calcium was determined by a colorimetric method.[30] Serum sodium, potassium, magnesium, and pH were determined by standard clinical methods.

Ventricular Function Group.—In a separate group of 20 dogs, the isolated coronary perfusion system was not used. In normal hearts, as left atrial pressure is increased, left ventricular work measured in kilogram-meters increases and then levels off.[23] The left atrial pressure may then be plotted against ventricular work to give a ventricular function curve. Cardiac outputs, used in the ventricular work calculation, were obtained prior to and after extracorporeal perfusion by dye dilution techniques.[14] Evans blue dye, a constant withdrawal syringe, and a cuvette densitometer were used. The instrument was calibrated daily by the volumetric technique of Fox.[10] Cardiac output determinations were made 10 minutes apart. During the cardiac output studies aortic and left atrial pressures were recorded. The left atrial pressures were varied between 6 and 26 cm. of H_2O by increasing or decreasing the blood volume.

Cardiac arrest was performed with each of the 3 methods under study in accordance with accepted clinical techniques. The chemical agents were mixed with blood and then rapidly injected into the aorta proximal to an occlusive clamp. Cold arrest was accomplished by the method developed in this laboratory.[26] Both atria were vented during the cold arrests, but only the left atrium was catheterized in the chemical arrests.

Myocardial oxygen tension was monitored before, during, and after the arrest by means of an open-end platinum microelectrode. This apparatus has been previously described by Davies and Brink and later by Montgomery and Horwitz.[20] The equipment was calibrated by insertion of the microelectrode into myocardium which was immersed in saline equilibrated with oxygen at various temperatures and partial pressures. All readings were then adjusted to 37° C. and calculated as a percentage of prearrest control readings.

The aortic clamp was released after the standard arrest. As soon as the coronaries were reperfused and the heart normothermic, defibrillation was undertaken with electric shock. Extracorporeal circulation was terminated, if possible, 15 minutes after the release of the aortic clamp. Fifteen minutes later postarrest ventricular function studies were initiated. Five to 10 mEq. of calcium gluconate was given to nearly all dogs postoperatively, but inotropic vasopressors (ephedrine and epinephrine) were used only when necessary to resuscitate the heart. No drug other than calcium was used in hearts arrested with cold.

RESULTS

The oxygen consumption of the exposed, normothermic, beating, nonworking heart averaged 3.7 c.c. per 100 Gm. heart muscle per minute. There was no difference in the oxygen consumption of the heart during systemic extracorporeal circulation or during isolated coronary perfusion. Furthermore, the rate of oxygen consumption remained stable during the entire hour of isolated coronary perfusion in control animals.

As the myocardium was cooled, the oxygen consumption decreased as shown in Fig. 1. The sharpest break in this curve occurred at approximately 30° C. Although the heart is mechanically and electrically arrested below 16° C., the slope of this curve does not change. At any specific temperature the oxygen consumption was the same whether the heart was being cooled or rewarmed.

All methods of cardioplegia produced a marked decrease in oxygen consumption as shown in Fig. 2. There was no significant statistical difference in oxygen utilization in chemically arrested hearts at 37° C. or 28° C. Both cold and potassium depressed oxygen consumption during arrest, but the rate of oxygen utilization during potassium cardioplegia was 2 and 3 times more than during cold arrest. Acetylcholine arrest was seldom complete since often the heart spontaneously contracted and always reacted to manipulation. These contractions probably account for the slightly higher oxygen utilization in these animals. With each method of cardioplegia the oxygen metabolism did not vary during the hour of arrest.

The oxygen consumption in the first half hour after termination of the arrest was significantly different in cold and chemically arrested hearts. After rewarming and defibrillation, the cold arrested hearts all resumed prearrest rates of oxygen consumption. In contrast, resuscitated chemically arrested hearts consumed oxygen at 50 per cent or less than control rates. The decreased utilization of oxygen after chemical arrest was seen in every experiment.

Studies with the platinum microelectrode in unperfused arrested hearts supplemented the metabolic studies (Fig. 3). In chemically arrested hearts

myocardial oxygen availability fell to 20 per cent of control values within 20 minutes of arrest. However, during cold arrest myocardial oxygen tension remained above 40 per cent of prearrest values for the duration of the arrest. Reperfusion of the heart resulted in a return to control values in all experiments.

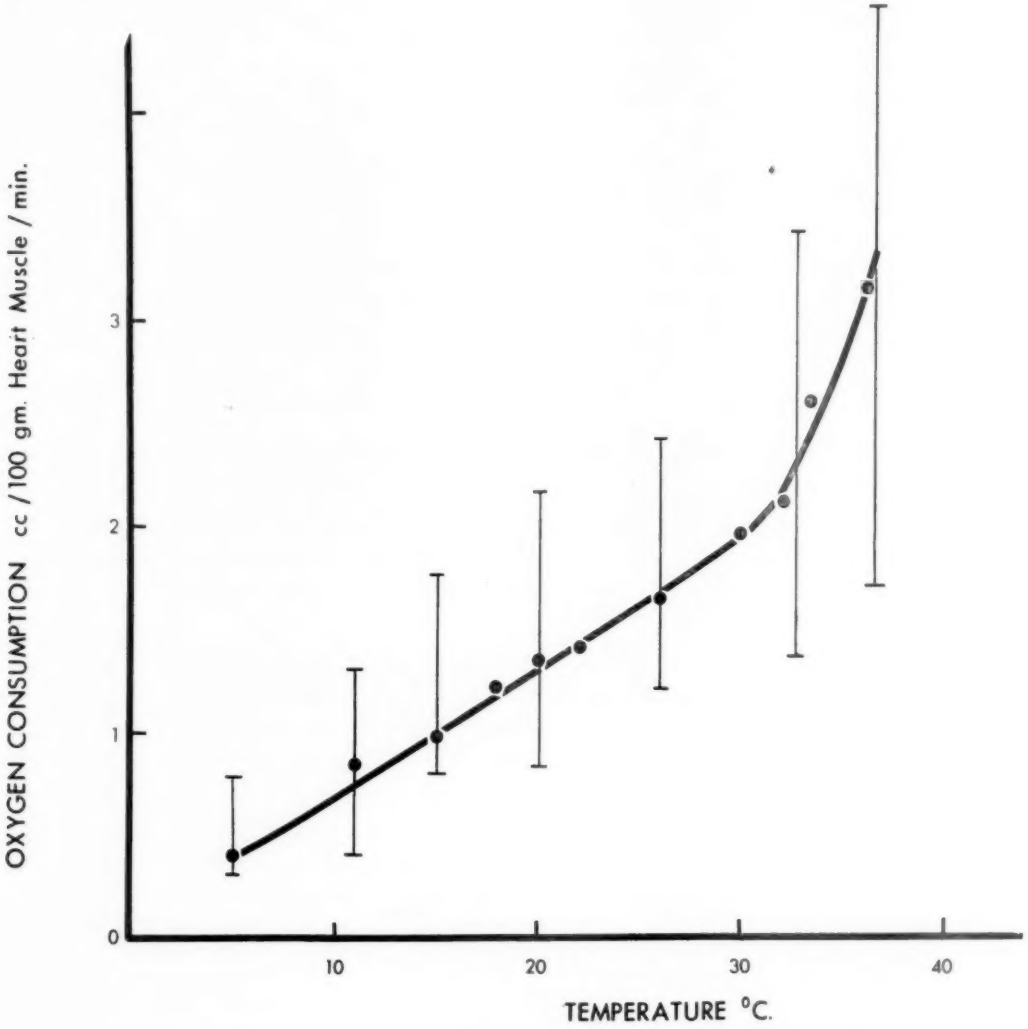

Fig. 1.—Oxygen consumption of the heart as a function of temperature. Each point is the average of 12 experiments.

The average coronary blood flow for each method of cardioplegia in the metabolic series is presented in Fig. 4. At the same perfusion pressure the coronary flow during chemical cardioplegia was greater than during cold arrest. Following cold arrest, coronary flow increased proportionately more than did postarrest oxygen consumption. Furthermore, in these experiments a consistent relationship between cardiac oxygen consumption and coronary blood flow could not be demonstrated.

There was no significant variation in serum sodium, calcium, magnesium, or pH during or after arrest with any of the methods of producing cardioplegia.

Serum potassium was normal during and after all arrests except when artificially elevated to produce potassium arrest.

Pre- and postarrest serum lactate determinations revealed no consistent differences between the cardioplegic methods. However, as recorded in Table I, all of the chemically arrested hearts produced more lactate during arrest than did the cold arrested hearts.

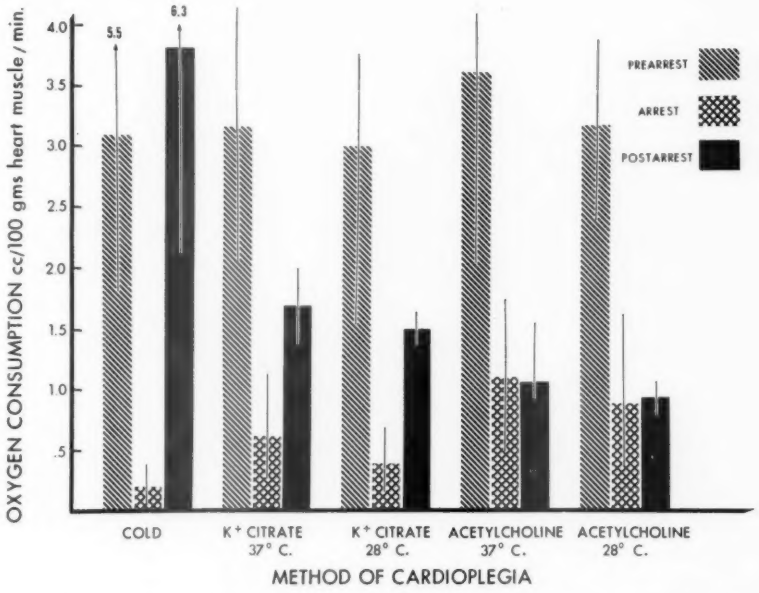

Fig. 2.—Myocardial oxygen consumption with cardioplegia. The 27 experiments were divided into 12 cold, 4 potassium citrate, and 3 acetylcholine at normothermia, 4 potassium citrate and 2 acetylcholine at 28° C., and 2 controls.

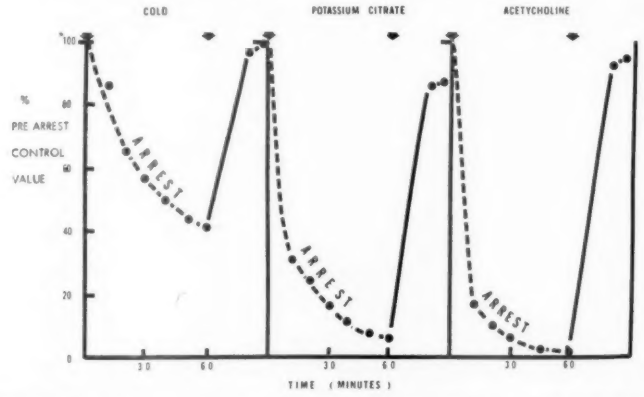

Fig. 3.—Myocardial oxygen availability in the arrested unperfused heart. Each point was calculated as percentage of the prearrest control value. Seven cold, 5 potassium, and 5 acetylcholine experiments were averaged.

The ventricular function curves before and after the standard arrest are graphically presented in Figs. 5 and 6. Postarrest left ventricular function or work capacity averaged only 16 to 29 per cent of prearrest control values after chemical cardioplegia. In contrast, left ventricular work capacity after cold arrest exceeded 80 per cent of prearrest values. Frequently, epinephrine and ephedrine or over 15 minutes of extracorporeal support were necessary to

resuscitate hearts arrested by acetylcholine or potassium. These aids were never necessary after cold cardioplegia, and the first left ventricular function study was performed one-half hour after release of the aortic clamp. When prolonged extracorporeal support was necessary, left ventricular function studies were initiated 15 minutes after the chemically arrested heart assumed the burden of the entire circulation.

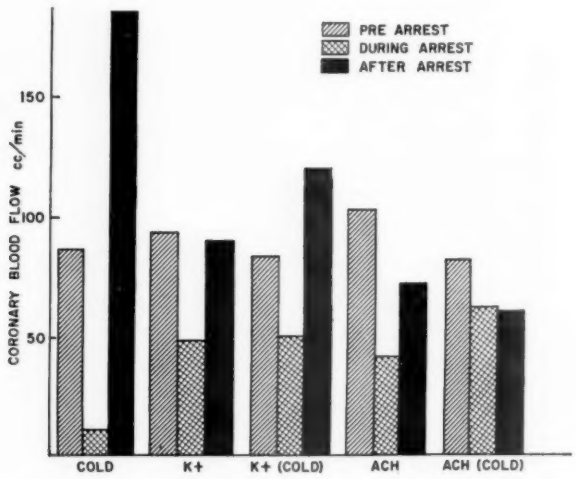

Fig. 4.—Average coronary blood flow with cardioplegia. The heart was perfused by an isolated extracorporeal system and perfusion pressure was maintained at 120 to 140 mm. Hg.

TABLE I. LACTIC ACID PRODUCTION DURING CARDIOPLEGIA

	COLD	K^+ 37°	K^+ 28°	ACH. 37°	ACH. 28°
Average	1.6 mg. %	12.3	23.9	15.2	9.8
Range	-6.7 to 11.5	6.0 to 16.4	14.3 to 33.6	8.3 to 22.2	4.8 to 14.8

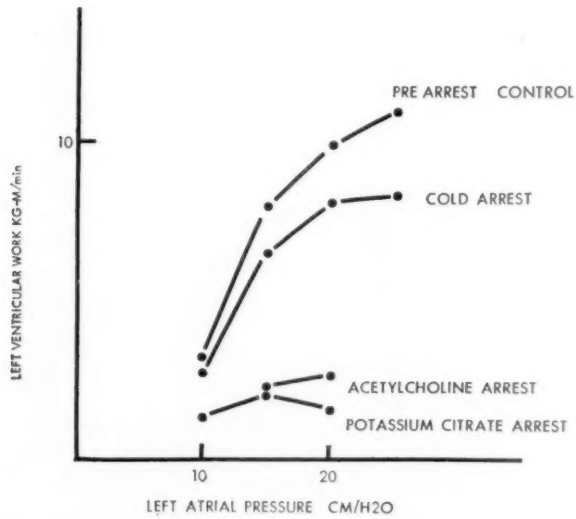

Fig. 5.—Left ventricular function following cardioplegia. The control curve has been plotted as a composite from the averaged prearrest ventricular work values of 20 dogs. Each postarrest curve was calculated as a percentage of its individual prearrest control. The postarrest curves for each type of arrest were averaged, and the average curve plotted as a percentage of the composite control.

Hematoxylin and eosin stained sections of the cold and acetylcholine arrested hearts did not reveal any abnormalities. Sections of the hearts arrested with potassium citrate showed areas of focal necrosis. Special stains and techniques were not applied in this study.

DISCUSSION

The mammalian heart can metabolize fatty acids, ketones, glucose, pyruvate, lactate, and probably amino acids.[5, 6] Fatty acids are the principal source of energy. Fat deposits and cardiac glycogen provide a reserve of metabolic substrates. The most critical factor is oxygen. Although the evidence is not conclusive, most investigators believe that the mammalian heart is capable of anaerobic metabolism.[6] However, the ability of anaerobic glycolysis to substitute fully for aerobic metabolism is notk nown. The duration of ischemia that can be tolerated by the heart at normothermia before it sustains irreversible damage is estimated to be about 20 minutes.[7, 11]

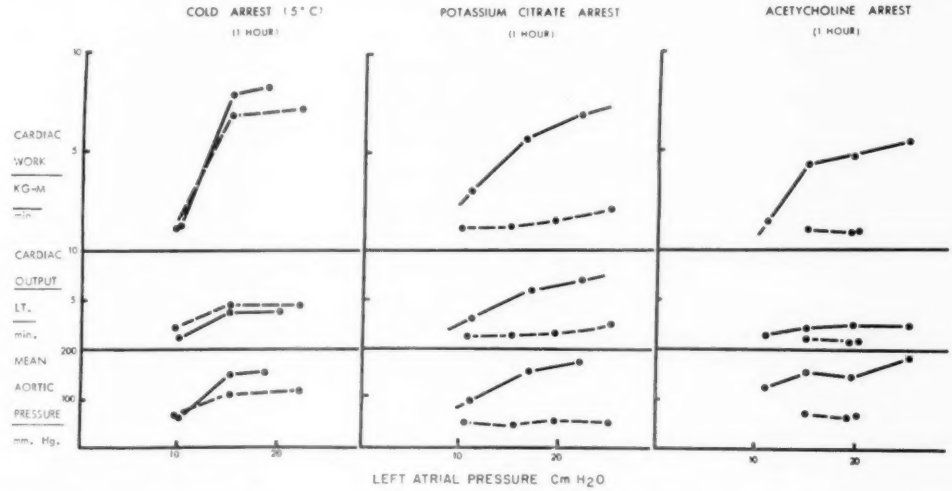

Fig. 6.—Representative ventricular function curves, cardiac outputs and aortic pressures in acetylcholine, potassium, and cold cardioplegia. The solid lines represent prearrest control values.

The oxygen consumption of the heart in the unanesthetized intact dog is 9.5 to 24 c.c. per 100 grams per minute.[25] During thoracotomy, however, the oxygen consumption of the working heart is only 5.6 to 13.7 c.c. per 100 grams per minute.[9] The control average of 3.7 c.c. of oxygen per 100 grams per minute for the exposed, beating, nonworking, normothermic heart in this study is in general agreement with previously published reports.[15, 28] The rate of oxygen consumption for the potassium arrested heart reported by McKeever[17] and Beuren,[4] is higher than the values found in this experiment and those published by Berglund.[3] These studies, however, are not entirely comparable since the experimental methods differed.

The basic objective of elective cardioplegia is to arrest the heart rapidly with a reversible method without producing myocardial damage. Potassium citrate and, to a lesser extent, acetylcholine rapidly stop the heart and have

proved reversible for short periods of arrest in large clinical series. The present study indicates that both acetylcholine and potassium decrease the oxygen utilization of the perfused arrested heart to approximately 1 c.c. per 100 grams per minute or less. However, the tissue oxygen data demonstrate that the unperfused, chemically arrested heart has very little interstitial oxygen available after 15 to 20 minutes of cardioplegia. This investigation also reveals that lactate is produced in significant quantities during 1 hour of chemical cardioplegia. These facts suggest that aerobic metabolism is markedly decreased with chemical cardioplegia, but nevertheless is significantly great so that these hearts consume nearly all the available oxygen and must resort to anaerobic glycolysis.

Following chemical cardioplegia the heart is unable to consume oxygen at prearrest rates. This decrease in utilization occurs in spite of adequate oxygen availability and implies that the metabolic machinery has been damaged. The marked depression of postarrest left ventricular function and the histologic changes noted in potassium arrested hearts reinforce this suggestion. Hence the conclusion must be entertained that chemical cardioplegic agents effecting arrest for 1 hour may damage cellular metabolism and structure.

Cold cardioplegia below 10° C. results in a very low rate of oxygen utilization and no appreciable lactate production in the perfused arrested heart. Since the number of experiments was limited, the difference in the rate of oxygen consumption during cold and chemical cardioplegia is not statistically significant. However, the greater interstitial oxygen availability in the cold arrested heart suggests that the rate of oxygen consumption was lower during this arrest.

The oxygen consumption after cold cardioplegia equalled control rates. This fact coupled with the markedly superior left ventricular function after cold arrest implies that the decreased metabolism during hypothermic arrest is largely reversible. Histologic studies have shown that the myocardium is not structurally injured after 1 hour of arrest at temperatures just above freezing.[26]

It is possible to freeze animal tissue without injury by using rigorous special techniques.[19] Usually, however, freezing destroys cells by intracellular crystallization or by dehydrating the cell. When a cell is dehydrated by freezing, the intracellular fluid becomes hypertonic and the cell proteins are gradually denatured. Cooling of intact cells to near 0° C. does not result in such cell damage and has been proved reversible even in the larger mammals.[1, 12] With cooling, both the metabolic needs and oxygen consumption decrease in parallel, so that during hypothermia aerobic metabolism is adequate for the energy requirements.[21] Extrapolation of the oxygen consumption curve (Fig. 1) indicates almost zero oxygen consumption at 0° C. Hence profound hypothermia to levels just above freezing would seem to provide the longest tolerance to ischemia. Work now in progress in this laboratory supports this hypothesis.

These experiments did not attempt to control the many variables that determine the rate of coronary blood flow. Only coronary perfusion pressure was controlled. Occasionally while the oxygen consumption, cardiac function, and coronary perfusion pressure remained constant, coronary blood flow varied as much as 60 c.c. per minute over a period of 5 minutes. Since the oxygen

consumption did not vary, a change in coronary flow required a reciprocal change in coronary arteriovenous oxygen difference. Thus these experiments with the isolated perfused heart during cardiac arrest did not demonstrate a correlation between coronary blood flow and oxygen consumption.

Since cardiac output was determined by dye dilution techniques, the calculation of left ventricular work in kilogram-meters per minute more fully reflects left ventricular work capacity than does calculation of stroke work. The difference in left ventricular function between hearts arrested with cold and those arrested chemically is remarkable. This study did not attempt to determine if the chemically arrested heart regained function in time. Since the greatest stress to the heart after cardiac operations usually occurs when the extracorporeal perfusion is stopped, the function of the heart in the immediate postarrest period is most critical. The satisfactory left ventricular function of the canine heart after cold arrest is corroborated in a small clinical series. These human hearts have been easily defibrillated and have reliably maintained the systemic circulation immediately after termination of up to 46 minutes of ischemic cold arrest. Furthermore, these hearts have not required inotropic vasopressors during the postoperative period.

This study compares 3 methods of producing cardioplegia in terms of myocardial metabolism and ventricular function. Since special histologic techniques were not employed, the pathologic studies are not definitive. The standard arrest was maintained for 1 hour because there is need for a safe method of cardioplegia for that period or longer. From this study it is concluded that for prolonged cardiac arrest cold cardioplegia below 10° C. is superior to acetylcholine or potassium cardioplegia.

SUMMARY

1. The oxygen consumption of the normothermic, nonworking, beating, canine heart is 3.7 c.c. per 100 grams heart per minute.

2. The oxygen consumption of the canine heart decreases sharply from 37° to 30° C. and more gradually from 30° to 5° C.

3. The oxygen consumption of the perfused arrested heart is 1 c.c. per 100 grams per minute or less during cold, potassium citrate, or acetylcholine cardioplegia. Following arrest the oxygen consumption of the chemically arrested hearts did not return to control values.

4. There is a consistent increase in lactic acid production during chemical arrest.

5. Myocardial oxygen availability in the unperfused heart is significantly higher during 1 hour of cold arrest than during chemical arrest.

6. Ventricular work capacity following cold arrest is substantially greater than that following chemical arrest.

7. This study indicates that for cardioplegia of 1 hour, cold arrest is superior to arrest by potassium citrate or acetylcholine.

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REFERENCES

1. Adolph, E. F.: Effects of Low Body Temperature on Tissue Oxygen Utilization, in The Physiology of Induced Hypothermia, Publication 451, National Academy of Sciences, National Research Council, Washington, D. C., 1956, pp. 44-49.
2. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, J. Biol. Chem. **138**: 535, 1941.
3. Berglund, E., Monroe, R. G., and Schreiner, G. L.: Myocardial Oxygen Consumption and Coronary Blood Flow During Potassium Induced Cardiac Arrest and During Ventricular Fibrillation, Acta physiol. scandinav. **41**: 261, 1957.
4. Beuren, A., Sparks, C., and Bing, R. J.: Metabolic Studies on the Arrested and Fibrillating Perfused Heart, Am. J. Cardiology **1**: 103, 1958.
5. Bing, R. J.: The Metabolism of the Heart, Harvey Lectures, p. 27, 1954.
6. Bing, R. J., Choudhury, J. D., Michal, G., and Kako, K.: Myocardial Metabolism, Ann. Int. Med. **49**: 1201, 1958.
7. Blumgart, H. L., Hoff, H., Landowne, M., and Schlesinger, M. J.: Experimental Studies on the Effect of Temporary Occlusion of Coronary Arteries in Producing Consistent Electrocardiographic Changes, Am. J. M. Sc. **194**: 493, 1937.
8. Davies, P. W., and Brink, F.: Microelectrodes for Measuring Local Oxygen Tension in Animal Tissues, Rev. Scient. Instruments **13**: 524, 1942.
9. Eckenhoff, J. E., Hafkenschiel, J. H., Foltz, E. L., and Driver, R. L.: Influence of Hypotension on Coronary Blood Flow, Cardiac Work and Cardiac Efficiency, Am. J. Physiol. **152**: 545, 1948.
10. Fox, S.: Personal communication.
11. Gollan, F., and Nelson, I. A.: Anoxic Tolerance of the Beating and Resting Heart During Perfusion at Various Temperatures, Proc. Soc. Exper. Biol. & Med. **95**: 485, 1957.
12. Gollan, F., Tysinger, D. S., Grace, J. T., Kory, R. C., and Meneely, G. R.: Hypothermia of 1.5 Degrees Centigrade in Dogs Followed by Survival, Am. J. Physiol. **181**: 297, 1955.
13. Helmsworth, J. A., Kaplan, S., Clark, L. C., Jr., McAdams, A. J., Matthews, E. C., and Edwards, F. K.: Myocardial Injury Associated With Asystole Induced With Potassium Citrate, Ann. Surg. **149**: 200, 1959.
14. Hetzel, P. S., Swan, H. J. C., Ramirez De Arellano, A. A., and Wood, E. H.: Estimation of Cardiac Output From First Part of Arterial Dye-Dilution Curves, J. Appl. Physiol. **13**: 92, 1958.
15. Jardetzky, O., Greene, E. A., and Lorber, V.: Oxygen Consumption of the Completely Isolated Dog Heart in Fibrillation, Circulation Res. **4**: 144, 1956.
16. Lam, C. R., Geoghegan, T., and Lepore, A.: Induced Cardiac Arrest for Intracardiac Surgical Procedures, J. Thoracic Surg. **30**: 620, 1955.
17. McKeever, W. P., Gregg, D. E., and Carney, P. C.: Oxygen Uptake of the Nonworking Left Ventricle, Circulation Res. **6**: 612, 1958.
18. Melrose, D. G., Dreyer, B., Bentall, H. H., and Baker, J. B. E.: Elective Cardiac Arrest, Lancet **2**: 21, 1955.
19. Meryman, H. T.: Mechanics of Freezing in Living Cells and Tissues, Science **124**: 515, 1956.
20. Montgomery, H., and Horwitz, O.: Oxygen Tension of Tissues by the Polarographic Method, J. Clin. Invest. **29**: 1120, 1950.
21. Penrod, K. E.: Cardiac Oxygenation During Severe Hypothermia in Dog, Am. J. Physiol. **164**: 79, 1951.
22. Reynolds, S. R. M., Kirsch, M., and Bing, R. J.: Functional Capillary Beds in the Beating, KCl Arrested and KCl Arrested-Perfused Myocardium in the Dog, Circulation Res. **6**: 600, 1958.
23. Sarnoff, S. J.: Myocardial Contractility as Described by Ventricular Function Curves; Observations on Starling's Law of the Heart, Physiol. Rev. **35**: 107, 1955.
24. Sealy, W. C., Young, W. G., Jr., Brown, I. W., Jr., Lesage, A., Callaway, H. A., Jr., Harris, J. S., and Merritt, D. H.: Potassium, Magnesium and Neostigmine for Controlled Cardioplegia, A. M. A. Arch. Surg. **77**: 33, 1958.
25. Spencer, F. C., Merrill, D. L., Powers, S. R., and Bing, R. J.: Coronary Blood Flow and Cardiac Oxygen Consumption in Unanesthetized Dogs, Am. J. Physiol. **160**: 149, 1950.
26. Urschel, H. C., Jr., and Greenberg, J. J.: Differential Hypothermic Cardioplegia, S. Forum **10**: 506, 1960.
27. Urschel, H. C., Jr., Greenberg, J. J., and Roth, E. J.: Rapid Hypothermia: An Improved Extracorporeal Method, J. Thoracic Surg. **39**: 318, 1960.
28. Van Citters, R. L., Ruth, W. E., and Reissmann, K. R.: Effect of Heart Rate on Oxygen Consumption of Isolated Dog Heart Performing No External Work, Am. J. Physiol. **191**: 443, 1957.

29. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, J. Biol. Chem. 61: 523, 1924.
30. Williams, M. B., and Moser, J. H.: Colorimetric Determination of Calcium With Ammonium Purpurate, Analytical Chem. 25: 1414, 1953.
31. Willman, V. L., Cooper, T., Zafiracopoulos, P., and Hanlon, C. R.: Depression of Ventricular Function Following Elective Cardiac Arrest With Potassium Citrate, SURGERY 46: 792, 1959.

DISCUSSION

DR. WILLIAM W. L. GLENN (New Haven, Conn.).—I hope that these important studies will be carried out with other cardioplegia techniques. Several techniques are now available that cause cardiac asystole as an aid in the performance of open heart surgery. Perhaps the oldest of these is that of induced ventricular fibrillation during cardiopulmonary bypass. With this technique it is essential, of course, that coronary flow be maintained. The disadvantage is that the coronary venous return must be removed from the open heart and that the blood sometimes obscures the field. We have used this technique of cardiac asystole in the approach to all four chambers of the heart for various cardiac defects, maintaining the heart in a state of continuous fibrillation for well over an hour in some cases. There has never been any difficulty in the prompt restoration of cardiac rhythm.

This slide is that of a patient with a left ventricular aneurysm pre- and postoperatively. I am sorry we missed seeing the ventricle before it was placed in fibrillation. The left ventricle is now being opened and the aneurysm is excised. As you can see in this part of the film the ventricles are in fibrillation. This patient also had complete heart block (and Stokes-Adams attacks) preoperatively with a ventricular rate of about 40. When we opened the chest, we placed an electrode in the wall of the right ventricle and stimulated the heart, in the manner reported by the Minnesota group, at a rate of about 80 to 90 per minute until we began cardiopulmonary bypass. We then induced and maintained ventricular fibrillation by passing 4 to 5 volts continuously into the right ventricle through the same wire electrode. This part of the film shows a mural thrombus being removed from the inside of the aneurysm. We now skip about an hour of this procedure when the excision was being completed and the left ventricle closed. The fibrillatory current was then discontinued and the heart shocked into a state of standstill with 120 volts. This is the shock being applied. The patient's heart promptly began to beat again but with a third degree A-V block. This was the rhythm before excision of the aneurysm. We again began to stimulate the heart at a rate of 80 to 90 per minute. Of great interest is the fact that 2 days following operation the third degree block reverted to a first degree block and the patient has not required stimulation since this time.

In children, when we stop induced fibrillation, the ventricle usually reverts to a normal rhythm spontaneously, but in the adult reversion usually requires a single shock to the heart.

DR. C. WALTON LILLEHEI (Minneapolis, Minn.).—Early in our experience with open cardiotomy we were troubled at times by the beating heart, and as a result described the value of anoxic arrest (SURGERY 38: 11-29, 1955). Recognizing that this represented a compromise between the needs of the surgeon and the needs of the myocardium for an uninterrupted supply of oxygen, we recommended that the arrest period be made as short as possible by intermittent occlusion rather than continuous clamping. This method worked satisfactorily and was used until potassium arrest was introduced in 1956. We tested this method and found it efficient for obtaining a motionless heart, but it was apparent that the morbidity and mortality rates were distinctly greater with chemical arrest (Minn. Med. 40: 672-676, 1957). This became most apparent in the patient who was a poorer risk preoperatively. Most cardiac surgeons have come to the same conclusion and potassium arrest is little utilized now.

However, we were not satisfied with anoxic arrest either, and experimental work continued eventuating in the development of selective cardiac hypothermia. Our studies in both animal and human hearts (S. Forum **10**: 544, 1959) indicate, as do the results presented today, that it is clearly superior. Clinically, since October, 1958, we have utilized selective cardiac hypothermia as the method of choice when elective arrest is desired.

To date, all of the described complications attributable to anoxic or chemical arrest have been acute. Today I should like to describe a late syndrome of myocardial anoxia. While this syndrome has not apparently been previously recognized, it is safe to predict that a large number of unfortunate cardiac cripples with varying degrees of this disability will be recognized, inasmuch as long periods of myocardial ischemia were enthusiastically advocated by many surgeons.

This report concerns a 14-year-old girl with the tetralogy of Fallot defects. Correction by open cardiotomy was undertaken in August, 1958. The heart was arrested for 31½ minutes with 45 c.c. of 2.5 per cent potassium citrate by the Melrose technique. The operation and hospital convalescence were uncomplicated. The girl had returned to school and appeared to have had an excellent result from surgical treatment. However, she was readmitted in November, 1958, with dilated heart and congestive heart failure of 3 weeks' duration. There were no cardiac murmurs, and thus the cause of the failure remained perplexing at that time. She responded to appropriate therapy and did fairly well until readmission in April, 1959, again in congestive failure. She responded to therapy and was discharged to be admitted for the fourth and final time in June, 1959. Despite intensive medical therapy for the chronic congestive failure, she died 12 days later. An autopsy was performed and was most revealing. The ventricular defect closure and excised infundibular area were completely healed. The pulmonary and aortic valves were normal. However, the diffuse pale appearance grossly of the entire myocardium was noteworthy. Diffuse, widespread fibrosis with only residual islands of viable muscle cells was evident. The coronary arteries were carefully examined and were patent throughout. A section of the myocardium excised from the infundibulum at the time of corrective surgery showed that all of the muscle cells had a completely normal architecture.

In summary, a patient survived corrective heart surgery for tetralogy of Fallot defects with a heart restored to normal anatomy but died 10 months later from chronic myocardial failure due to diffuse myocardial fibrosis apparently secondary to a 31-minute period of ischemia during potassium citrate arrest.

DR. EDMUNDS (closing).—In reply to Dr. Glenn's discussion we are presently evaluating myocardial oxygen availability and postarrest left ventricular function in hearts arrested by ischemia for varying periods. We have not evaluated chemical methods of cardioplegia other than acetylcholine and potassium.

Dr. Glenn has suggested inducing ventricular fibrillation during extracorporeal perfusion for the repair of intracardiac lesions and ventricular aneurysms. While feasible for many defects, this method is not applicable for aortic valvular surgery. We prefer cardiac arrest for the repair of either aortic or ventricular septal lesions.

Dr. Lillehei's finding of myocardial fibrosis and cellular necrosis months after potassium arrest supplements our acute observations and those of others. On the basis of histologic evidence and the accumulating metabolic, functional, and clinical data, it may be concluded that cold arrest is superior to chemical cardioplegia.

OPERATIVE APPROACH TO TRANSPOSITION OF THE GREAT VESSELS

I. Classification and Review of 32 Cases With and 40 Cases Without Operation

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COMPLETE transposition of the great vessels is one of the more common anomalies with which cyanosis is associated. It is defined as a reversal of the sites of origin of the aorta and pulmonary artery so that the aorta arises from the right ventricle anteriorly and the pulmonary artery from the left ventricle posteriorly. Included in the definition, according to Lev,[18] should be those cases of common (single) ventricle where the aorta arises anterior to the pulmonary artery.

Survival depends on the intermixing that is promoted between the pulmonary and systemic circulations by a communication between left- and right-side cardiac chambers. Death results before 6 months in more than 50 per cent of untreated patients because of anoxia or cardiac decompensation (Fig. 1).

Operative cure of this anomaly consists essentially of redirection of the blood flow to enable systemic venous blood to reach the lungs and arterialized blood the periphery, with correction of septal defects and other associated cardiovascular anomalies at the same time. Complete correction—of the defect as well as the transposition—has been achieved in only 1 instance,[14, 25, 26] though a number of attempts have been made by various methods.[1, 2, 8, 9, 12, 15, 19-21, 23, 24]

Palliative procedures that make possible an extensive intermixing of blood from both circulations may prolong life until such time as a corrective procedure can be attempted. These palliative procedures have met with varying degrees of success, in many cases general improvement of the patient.[4, 5, 7, 11, 28]

It is the purpose of this paper to review the clinical features, laboratory data, operative experience, and postmortem findings in 72 cases of transposition seen in the Yale–New Haven Medical Center over the past 13 years and, on the basis of this review, to make some recommendations or reasonable hypotheses for the surgical management of this condition.

The review has emphasized the need for a detailed consideration of associated defects in the selection of operative procedures. Accordingly, we have established the following classification:

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*U. S. Public Health Research Fellow.

CLASSIFICATION OF TRANSPOSITION
ACCORDING TO ASSOCIATED INTERCOMMUNICATIONS

Group I.—
- A. Patent foramen ovale (or atrial septal defect) (small)
- B. Patent foramen ovale (or atrial septal defect) (large)
- C. ± other associated defects:
  Patent ductus arteriosus

Group II.—
- A. Ventricular septal defect (small)
- B. Ventricular septal defect (medium)
- C. Ventricular septal defect (large, single or common ventricle)
- D. ± other associated defects:
  Patent foramen ovale
  Patent ductus arteriosus
  Mitral or tricuspid stenosis or atresia

Group III.—
- A. Pulmonary stenosis or atresia and ventricular septal defect (small or medium)
- B. Pulmonary stenosis or atresia and ventricular septal defect (large, single or common ventricle)
- C. ± other associated defects:
  Patent foramen ovale
  Patent ductus arteriosus

Group IV.—
- A. Coarctation
- B. Hypoplasia, right heart
- C. Atrioventricular cushion defects
- D. Others
- E. ± other associated defects:
  Patent foramen ovale
  Ventricular septal defect
  Patent ductus arteriosus

GROUP I

There were 18 patients with intracardiac communication at the atrial level, 17 of whom came to postmortem examination, and one of whom is still living. In 16 of these the defect was confined to the region of the foramen ovale. In 1, almost all of the atrial septum was absent. The flap of the foramen ovale was complete in 8 cases and incomplete in the other 8 cases. In 14 of 17 cases the ductus was patent, ranging in diameter from 1 to 3 mm. The length of life in the patients in this group was seemingly related to the size of the atrial defect. For patients with the small defect the average duration of life was 17 days with a range of 1 to 48 days and for those with the large defect the average duration of life was 135 days with a range of 3 days to 27 months.

Clinical Features (Postmortem-Proved Cases).—Progressive cyanosis was seen in all patients except in the infant with the large atrial defect. In 3 patients just prior to death, the lower half of the body appeared to be less cyanotic than the upper, suggesting the presence of a pulmonary artery–systemic artery shunt through a patent ductus. Growth was markedly retarded.

A precordial systolic murmur was noted in most of the cases, and there was sometimes an associated thrill. Cardiac decompensation was present in 14 cases. No signs of failure and no pulmonary congestion were noted in 3 cases and in these the ductus was closed at autopsy. The electrocardiogram was nonspecific with right axis deviation in every instance.

On roentgen examination increased pulmonary vascular markings were noticeable in every patient beyond the age of 1 week with the exception of 2 in whom autopsy subsequently proved the existence of an obliterated ductus. In 1 patient with a widely patent foramen ovale the radiopaque dye could be seen by cineangiography to move freely back and forth—to the left during atrial systole and to the right during atrial diastole—through the foramen ovale.

Cardiac catheterization was performed in only 1 patient in this group. To broaden this study we have included in Table I autopsy-proved Group I patients of Fowler and Ordway[10] who had undergone catheterization studies. It is apparent from these studies that a shunt at the atrial level, even through the small defect, is bidirectional. There was no evidence, from the oxygen content of blood samples taken from the right ventricle and the femoral artery simultaneously, of a shunt from the pulmonary artery to the aorta. But a right ventricular pressure that was found to be higher than the left indicated that any

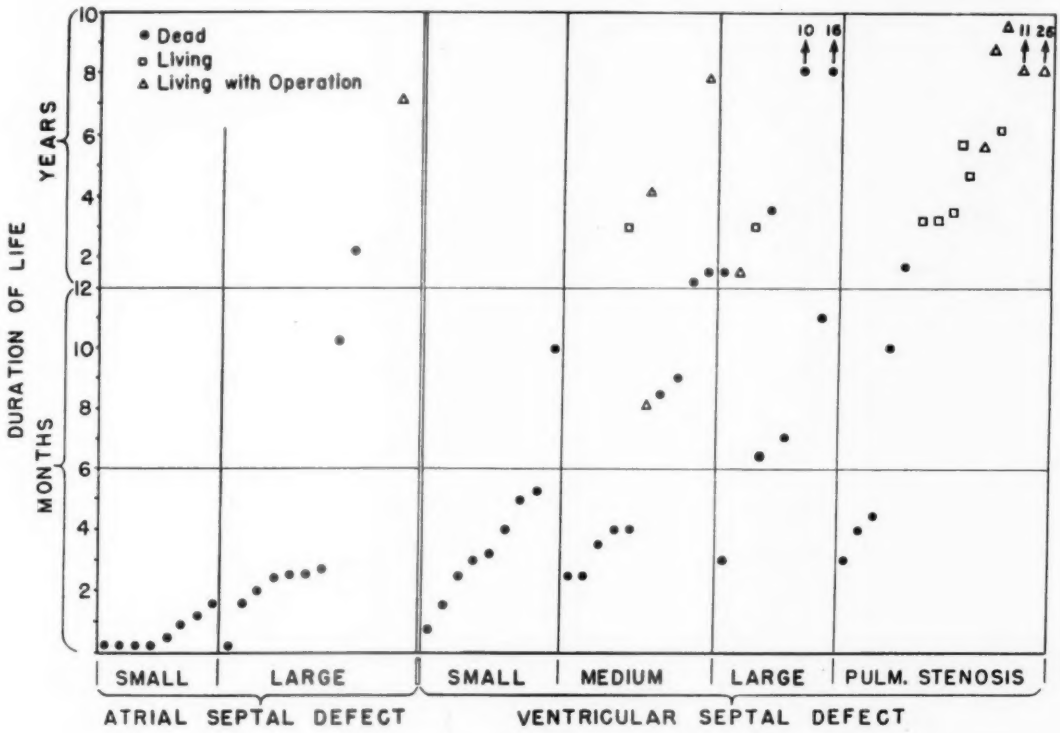


Fig. 1.—Range of survival in Groups I, II, and III.

shunt through a patent ductus would have been from the aorta to the pulmonary artery. Histologic examination of the pulmonary vasculature in 12 patients in this group showed only the normal architecture or fetal type vessels except in the 1 patient who survived for 27 months. In this patient grade 3 changes were found (by the histologic classification of Heath and Edwards[13]).

Treatment.—An operation was performed in 7 cases. Only 2 patients survived to leave the hospital, both of whom had atrial septal defects created by the technique described by Blalock and Hanlon.[6] One had, in addition, an end-to-end anastomosis of the right subclavian artery and right pulmonary artery. This patient died in congestive failure about 6 months later.

Two patients were operated upon at 9 and 42 days of age, respectively, with the mistaken diagnosis of pulmonary stenosis, made on the basis of the clear lung fields. An aortic–pulmonary artery shunt was made in both with death at operation in the older patient and after 39 days in the younger patient as a result of cardiac decompensation. The

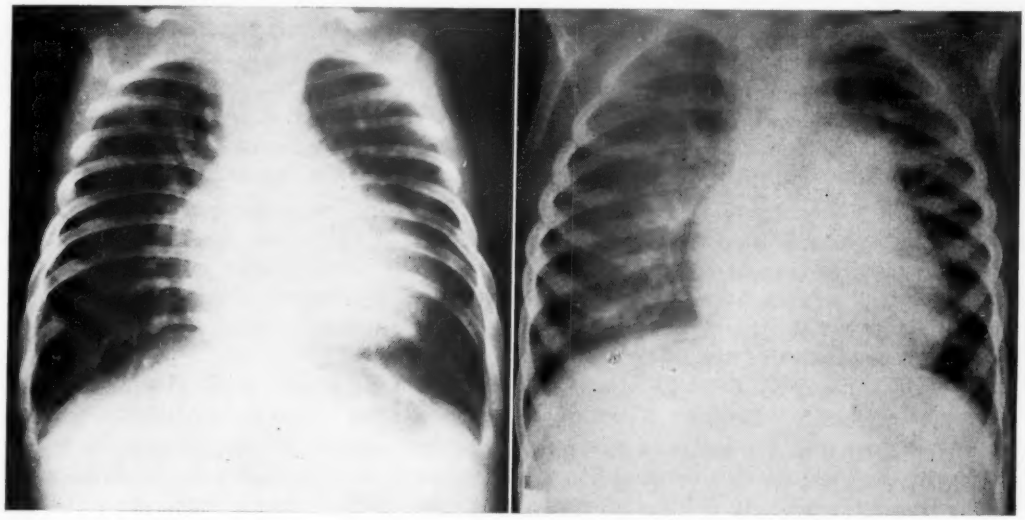
TABLE I. OXYGEN CONTENT DATA OBTAINED BY CARDIAC CATHETERIZATION IN TRANSPOSITION OF THE GREAT VESSELS, GROUP I PATIENTS

POSITION OF CATHETER	4-DAY-OLD PATIENT*			3-WEEK-OLD PATIENT*			4-WEEK-OLD PATIENT			5-WEEK-OLD PATIENT*		
	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE
Superior vena cava	4.7			3.5			1.2			7.7		
Left atrium			9/–2			8†			11/8			7/1
Near pulmonary vein										26.4	100	
Near defect	16.4						11.5					
Midatrium	19.9	95		14.8	94		14.7	86				
Pulmonary vein	22.3	100		17.1	100					26.5	100	
Right atrium			6/–2			8†			9/6			1/0
Midatrium	8.4			8.9	57		2.4			5.2		
Low atrium							1.1					
Left ventricle			39/1			21/10‡						20/0
Near mitral valve	20.5											
Midventricle	19.3			11.7	74					24.4	97	
Right ventricle	6.4§‖		78/0	8.3	53	43/10‡				6.6§		60/3
Femoral artery	6.5§‖		70/55			48†	2.4	13	75/44	6.7§	27	76/50
Oxygen capacity	20.8			15.5			16.8			23.3		
Postmortem exam.	PFO			PFO, ASD, PDA			PFO, PDA			PFO, PDA		

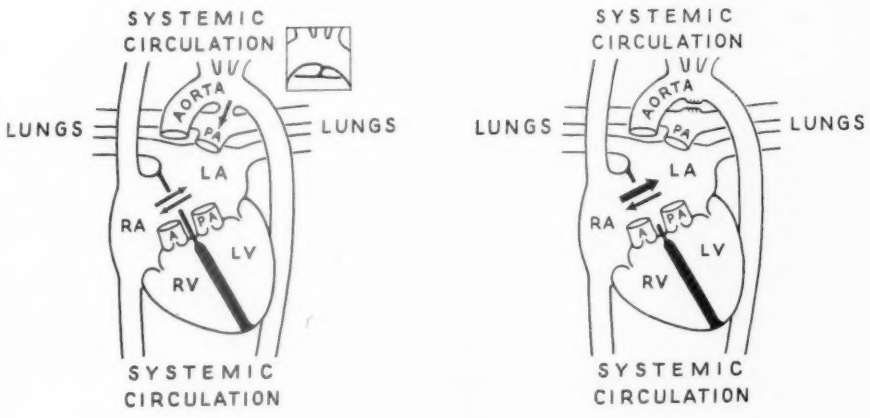
*Entire procedure performed while child received 100 per cent oxygen by mask.
†Mean pressure.
‡Complete heart block present.
§Samples removed simultaneously from right ventricle and femoral artery.
‖Child crying when samples removed.

ductus arteriosus was closed in both patients at postmortem examination. The 1 survivor in this group, now 7 years of age and suffering progressive limitation, is now ready for an attempted total correction (Fig. 2).

Comment.—It would appear obvious from the foregoing that patients with transposition cannot survive for more than a few days if mixing of the systemic and pulmonary circulations is limited by the small opening in the foramen ovale. A totally corrective procedure for transposition is not possible by present techniques during the first weeks or months of life, the time when nearly all of the Group I patients require help.



A. *B.*



C. *D.*

Fig. 2.—Transposition: Group I. Patent foramen ovale. *A*, Roentgenogram of a 5½-month-old child with transposition who had an atrial defect created by the Blalock-Hanlon technique at this time. The ductus was not explored. *B*, Roentgenogram showing increased pulmonary vascular markings at age 7 years. Catheterization and angiography were interpreted as demonstrating a communication at only the atrial level. *C* and *D*, Illustration of the direction of shunts and proposed palliative procedure in Group I patients.

The patient who survived the longest in this group—without operation—had a large patent foramen ovale, the bidirectional shunt clearly demonstrable by cineangiography, and no patent ductus demonstrated at the time of this examination (age 19 months) or at autopsy at age 27 months. The 1 living patient in this group had an atrial septal defect created at age 5 months, and from recent catheterization and angiocardiography studies, at age 7 years, it is believed that the only shunts are at the atrial level. Presumably better

intermixing of systemic and pulmonary blood at the atrial level is obtained in the presence of a large defect. Two patients, aged 3 and 2½ years, were reported by Lenkei, Swan, and DuShane[17] as having only an atrial defect with a "substantial degree of mixing." The patient operated on by Senning[25] was 9 years of age and had only wide (17 mm.) patency of the foramen ovale.

There is nothing to suggest from a review of our cases that patency of the ductus was beneficial to the patients in Group I. Patency of the ductus allows for flow in 1 direction only—from aorta to pulmonary artery—except in terminal cases. This may be harmful since severe pulmonary congestion results if return of the shunted blood from the pulmonary circulation back to the systemic circulation is restricted. Invariably, the infants who survived beyond the first week and who had a patent ductus showed marked pulmonary congestion by roentgenogram though it was interesting that we could not demonstrate by catheterization data an increased pulmonary flow in Group I patients during the first few weeks of life (Table I). The 1 older (living) patient probably does demonstrate an increased pulmonary flow. Keith[16] and Astley and Parsons[3] have commented on the better prognosis that is obtained by those patients in whom there is a closed ductus.

Thus, it seems apparent that a logical palliative procedure in Group I is (1) enlargement of the communication between the left and right atria to provide better mixing of systemic and pulmonary blood, and (2) interruption of a patent ductus, even though small, to prevent further pulmonary congestion. Better mixing of blood at the atrial level can be accomplished through one of several procedures: (1) enlargement of the atrial septal defect, (2) transposition of the inferior cava to the left atrium and the right pulmonary veins to the right atrium,[4] or (3) anastomosis of the superior vena cava to the distal right pulmonary artery and transposition of the right pulmonary veins to the right atrium.

We believe it may prove to be advantageous to interrupt the ductus and promote further mixing of the systemic and pulmonary circulations at the same operation. It is advisable not to expose the lungs if possible because of the tendency in these patients to focal edema and hemorrhage from manipulation—although this is less if there is temporary occlusion of the pulmonary artery to the lung. The sternum-splitting incision will allow exposure of the right atrium and the ductus. We have preferred the Blalock-Hanlon[6] technique for the creation of an atrial septal defect but have not done this through a midsternal approach. Using a midsternal approach we have enlarged the opening in the foramen ovale with an instrument through the atrial wall with digital guidance.

GROUP II

There were 32 cases of transposition with ventricular septal defect and no pulmonary stenosis. Twenty-six of these were examined post mortem. The 6 patients who are still living will be discussed in a separate section. In the autopsy-proved cases there were 24 of 26 with patent foramen ovale and 8 of 26 with patent ductus arteriosus. In 9 cases the ventricular septal defect was 5 by 2 mm. or less in size and in another 9 the size ranged from 5 by 2 to 5 by 10 mm. In 8 there was a functional single ventricle, 4 of which were associated with tricuspid or mitral stenosis or atresia. The length of life of these patients was seemingly related to the size of the ventricular septal defect. The average was 4 months in cases of small defect (range 24 days to 10 months), 7 months when it was of medium size (range 25 days to 18 months), and 3¾ years when there was a single ventricle (range 97 days to 16 years).

Clinical Features (Postmortem-Proved Cases).—Cyanosis was observed in 25 of 26 cases either at birth or shortly thereafter. There was a precordial systolic murmur often associated with a thrill and a diastolic murmur was rarely heard. Cardiac decompensation occurred within the first month of life in 7 patients, 2 of whom had a single ventricle. Growth was retarded.

The electrocardiogram was inconclusive, illustrating right axis deviation in about two-thirds of the cases of small and medium-sized defects. Left ventricular hypertrophy, a rare

finding in cases of transposition, was present, however, in 2 patients with a small ventricular septal defect and a patent ductus arteriosus.

Roentgen examination usually revealed cardiac enlargement and an increased pulmonary vasculature, findings similar to those in Group I. Angiocardiography, when performed, determined the relative position of the aorta and usually demonstrated a shunt at both atrial and ventricular levels.

Cardiac catheterization studies were carried out in 5 cases (Table II). A shunt was demonstrated at the atrial level. In 4 of the 5 there was a left-to-right shunt at the ventricular level. (The shunt from right-to-left at the ventricular level was shown in 10 of 11 cases by angiography.)

The pulmonary vasculature was examined microscopically in 18 cases. In 3 no microscopic changes were observed. In the remaining 15, changes ranging from grades 1 to 6 were found. Grade 6 changes were noted in 2 patients who were aged 10 and 16 years. Changes in the remaining 13 ranged from grades 1 to 3. Grade 3 changes were found in 5 patients of ages from 2½ to 20 months and in 1, aged 3½ years, whose anomaly was further complicated by a moderate infundibular stenosis of the left ventricle. A more detailed morphologic evaluation will be the subject of a subsequent report.

Treatment.—Operation was performed on 19 of 32 patients. Only 6 survived the immediate postoperative complications. One died 12 months after creation of an atrial septal defect, during a second similar operation. Another died 11 months after interruption of the pulmonary circulation to the left lower lobe (Vidone-Liebow[28]). Thirteen patients died in the early postoperative period. In none of these was banding of the pulmonary artery carried out.

There are 6 patients still living. These, we believe, have a ventricular septal defect in addition to the transposition. The average age in this group is 4½ years (range 8 months to 7 years 10 months). Four patients have had palliative surgical treatment, 2, creation of an atrial septal defect, and 2 others (in severe pulmonary edema), banding (constriction) of the main pulmonary artery[7, 22] (Fig. 3). The 2 patients with an operative atrial septal defect, now 6 and 7 years old, are awaiting more definitive diagnosis from catheterization and repeated angiocardiographic studies before undergoing a corrective procedure. Hopefully, the 2 patients with banding can be deferred for several years more before any further surgical treatment, corrective or palliative, is considered. One patient, now 4 years old, with catheterization and angiocardiographic studies indicative of a ventricular defect of moderate size, is presently awaiting a corrective procedure—reconstruction of the atrial septum and closure of the ventricular defect. The sixth patient is 3 years of age and has been shown to have a single ventricle. The plan at present is banding of the pulmonary artery followed with a vena caval-pulmonary artery shunt.

Comment.—As in Group I there are 2 basic problems, first, the need for thorough intermixing of blood from the pulmonary and systemic circulations and, second, the need for control of pulmonary congestion. It is apparent from the data presented that the larger the ventricular defect the longer the survival. This is undoubtedly due to a better intermixing of systemic and pulmonary blood. The severe degree of pulmonary congestion seen in these patients is the result of a shunt predominantly right-to-left at the ventricular level. There was an increased pulmonary flow in this group as shown by the catheterization data except in the oldest patient with advanced pulmonary vascular changes (Table II). Also contributory may be the shunt through the ductus when it is patent from the aorta to the pulmonary artery, unless the pulmonary artery pressure equals or exceeds the aortic pressure. The changes seen in the pulmonary arterioles after several months are believed to be secondary to an increased blood flow at high pressure.

Palliative operative treatment in Group II must be directed toward improving the intermixing of blood in the heart and reducing pulmonary congestion. In the cases of single ventricle and large ventricular defects intermixing of blood is probably maximal. In these patients, a reduction of pulmonary flow and pressure is indicated to possibly prevent irreversible pulmonary arterial and arteriolar damage. In view of the vascular changes, seen as early as 2½ months of age in 1 patient, banding of the pulmonary artery should be

TABLE II. OXYGEN CONTENT DATA OBTAINED BY CARDIAC CATHETERIZATION

	8-MONTH-OLD PATIENT			3½-YEAR-OLD PATIENT			13-MONTH-OLD PATIENT		
POSITION OF CATHETER	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE
Superior vena cava	0.8			9.5			7.7		
Left atrium Midatrium				21.4		16/8	23.9		5*
Pulmonary vein				22.0	91		25.8	86	
Right atrium Midatrium	2.2		2*	10.9		15/5	7.3		6*
Left ventricle									
Right ventricle (outflow)	4.5 2.7		40*	10.1 12.5		200/4	6.5		50*
Femoral artery	2.6 9.9‡	50		13.3	55	188/134	12.7 19.38‡	62	
Oxygen capacity	17.8			24.0			29.7		
Postmortem exam.	Medium VSD, PFO, PDA			Large VSD, PFO			Medium VSD, PFO		

*Mean pressure.
†Pulmonary artery.
‡Samples taken when child breathing ozygen.

performed as soon as the diagnosis is made. Further, it would seem logical to divide the ductus arteriosus if patent. If a small or medium-sized ventricular defect is suspected or demonstrated and intermixing of the blood can be improved, the creation of an atrial defect would seem to be a reasonable procedure at the same time.

Correction of the transposition may be attempted in certain patients in Group II who survived for some years—for technical reasons the age of 8 or 10 would seem ideal. Patients with small or medium-sized ventricular defects are the most likely candidates. This defect should be closed at the time of correction of the transposition. If the pulmonary artery has been banded, this would also require reconstruction.[27] A single ventricle is probably not correctable by present techniques. After the pulmonary artery has been banded, patients with a single ventricle should be considered candidates for an anastomosis of the superior vena cava to the right pulmonary artery (Group III).

GROUP III

Sixteen patients were classified as having pulmonary stenosis. Three of the 5 patients who died had atresia of the pulmonary valve and hypoplasia of the pulmonary artery. Severe pulmonary stenosis was found in the other 2. The ductus was patent in 3 of the 5 patients. A single ventricle was present in 4 of the 5 patients who died. The length of life in the 5 who died averaged 8 months (range 3 to 20 months). The 11 living patients averaged 8 years in age (range 3⅓ to 26 years).

Clinical Features (Postmortem-Proved Cases).—Cyanosis which was progressive was present in all 5 patients. A precordial systolic murmur was also heard in every case but it was of less intensity in the patients with atresia of the pulmonary valve. Cardiac decompensation was not seen in any instance. Anoxia, as manifested by apneic spells, dyspnea, and fatigability was the chief cause of distress. Patency of the ductus appeared to extend the life of the 2 patients who were the oldest survivors (10 and 20 months).

Roentgen examination demonstrated strikingly clear lung fields with little or no cardiac enlargement. Dextrocardia of the heart was shown in 2 patients. The electrocardiogram

IN TRANSPOSITION OF THE GREAT VESSELS, GROUP II PATIENTS

5-YEAR-OLD PATIENT			6-YEAR-OLD PATIENT			SAME PATIENT AT 9 YEARS		
VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE	VOL. %	PERCENTAGE SATURATION	PRESSURE
11.9			15.5			12.2		
						27.3	91	
13.0		0/–4	16.4		18	16.5		
20.2		120/57†				20.5†		
19.4		107/8	22.5		80	21.5		
19.3								
16.5			19.8			18.9		
19.1‡	82		23.6‡	72		22.8‡	76	
23.2			30.5			29.9		
	SV, PDA			SV				

showed progressive development of S waves across the precordium in 2 patients with levocardia and single ventricle. Cardiac catheterization was not performed in any of the 5 patients. Histologic examination of the pulmonary vasculature showed no changes in the 2 patients, aged 3 and 10 months.

Living Patients.—Eleven patients are living in various states of health. All exhibit cyanosis which was mild in some cases during infancy but which is extreme in the older patients. All have a systolic murmur sometimes accompanied by a thrill. Easy fatigability and dyspnea on exertion without evidence of cardiac decompensation but with moderate retardation of growth characterize this group.

The electrocardiogram shows right ventricular hypertrophy in 4 and a pattern suggestive of single ventricle in 3. Q waves were absent over the left precordium in 9. Two patients had dextrocardia without chamber inversion.

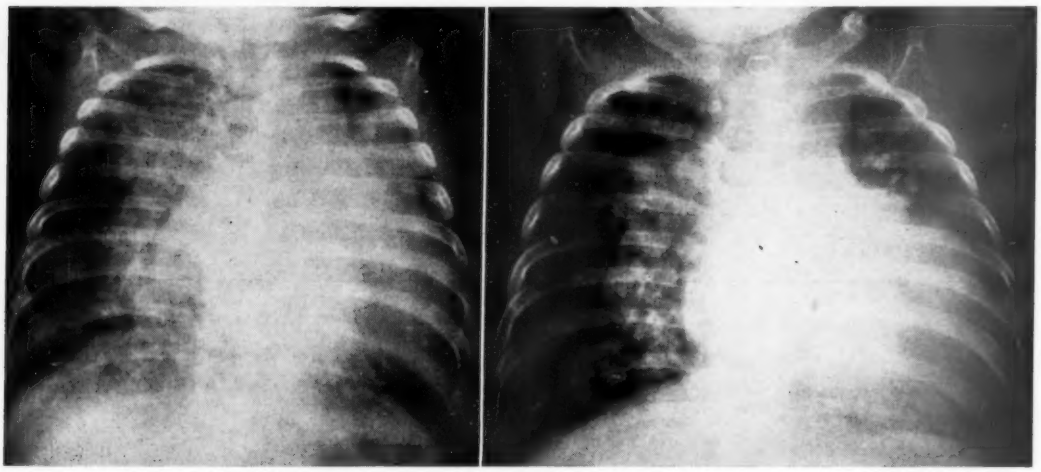
Roentgen examination showed the heart frequently to be of normal size. Some unique contours were observed in these hearts because of the ascending aorta forming the left upper border of the cardiac silhouette in some of the patients with single ventricle. In 2 patients there was dextrocardia. Angiography was performed in all cases confirming the anterior placement of the aorta and the presence of an intracardiac shunt. The lung fields were clear but pulmonary stenosis could not be demonstrated.

Cardiac catheterization was performed in 8 instances and in 7 demonstrated a defect at the ventricular level. In no case could the catheter be passed into the pulmonary artery. As a result of angiocardiography and cardiac catheterization a ventricular defect was demonstrated in every case and in 6 there was believed to be a single ventricle.

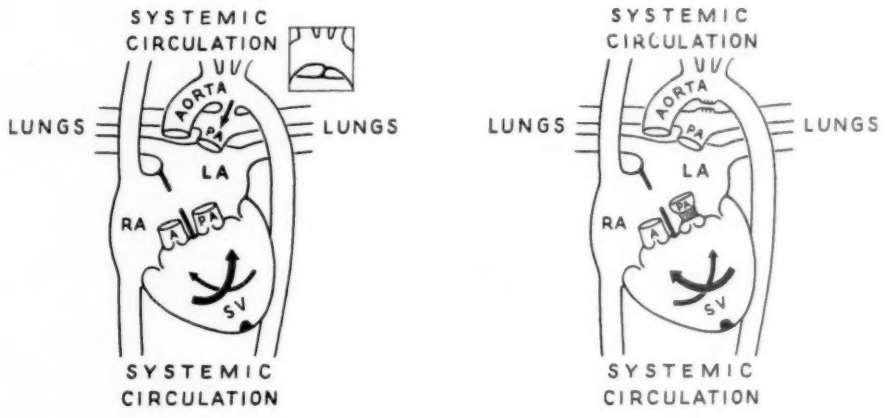
Treatment.—Five patients in this group have been operated upon—1 infant, aged 4 months, with pulmonary atresia was operated on but did not survive with an aorta–pulmonary artery anastomosis. Two have undergone the creation of an atrial septal defect (1 was later followed by a systemic to pulmonary artery shunt) and are now 4 and 5 years post operation. Two other patients have undergone an anastomosis performed between the side of the superior vena cava and the distal end of the right pulmonary artery (Fig. 4). One of these has been previously reported.[11] Both are improved 1 and 2 years postoperatively. Six patients in this group are now awaiting vena cava–pulmonary artery anastomosis.

Comment.—It is not possible to be certain that pulmonary stenosis is present in the living patient although the clinical features are compatible with this diagnosis. It has not been possible to pass a catheter into the pulmonary artery or to demonstrate the valvular stenosis by venous angiography. More precise diagnostic techniques may allow a demonstration of the stenosis in future investigations and may also cause us to revise the classification of some of these patients.

One patient, aged 26, deserves special comment. She did not seek medical care until she was 12 years old and the diagnosis of transposition was not made until recently when, during pregnancy, she experienced signs of mild cardiac failure. Repeat diagnostic studies on the Medical Service revealed the aorta definitely to be transposed. There is also a functional single ventricle, and the lung fields, clear since she was first seen 14 years ago, suggest the presence of pulmonary stenosis.



A. *B.*



C. *D.*

Fig. 3.—Transposition: Group II. Ventricular septal defect. *A*, Roentgenogram of a 5½-month-old child in intractable heart failure. Angiography confirmed the diagnosis of transposition and demonstrated a large ventricular septal defect. *B*, Roentgenogram 5 months after banding of the pulmonary artery. Clinical improvement was marked. Oxygen saturation and carbon dioxide tension studies suggest lessening of pulmonary congestion (Table III). *C* and *D*, Illustration of the proposed palliative procedure in Group II patients.

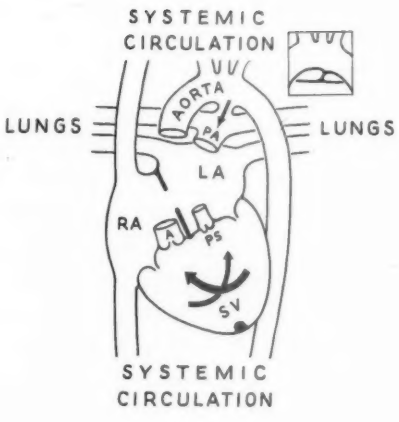
TABLE III. ARTERIAL STUDIES BEFORE AND AFTER BANDING OF THE PULMONARY ARTERY

BREATHING AIR WHILE CRYING	BEFORE OPERATION (5 MO. OF AGE)	AFTER OPERATION (10 MO. OF AGE)
O_2 sat. (%)	45	61
pCO_2 mm. Hg	0.74	0.49

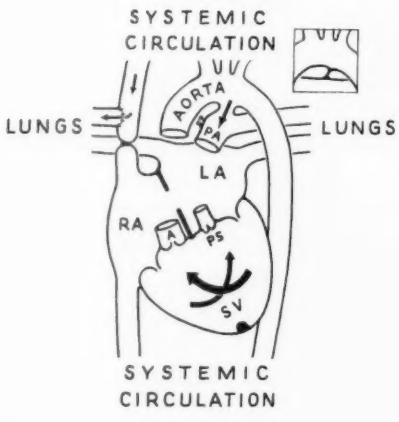
In Group III, the ventricular defect—frequently a single ventricle—encourages mixing of the blood between the 2 circulations and pulmonary stenosis protects the lungs from excessive blood flow at increased pressure. Thus we have the conditions, short of complete correction, that are apparently required in patients with transposition for survival for prolonged periods. In those patients with a ventricular defect of small or moderate size complete correction of the transposition should be considered. However, it is anticipated that there will be difficulty in reconstruction of the atrial septum, closure of the ventricular septal defect, and correction of the pulmonary stenosis (which may be infundibular) at the same operation.

We appreciate the fact that patients with a single ventricle present the same hemodynamic alteration regardless of the position of origin of the aorta and pulmonary artery from the common chamber. But from the standpoint of surgical repair there may be some advantage in making a distinction between the single ventricle with and without transposition. In the large ventricular septal defect, which is functionally a single ventricle, there may be sufficient septal tissue remaining for definitive repair. In such a case the

A. *B.*



C.



D.

Fig. 4.—Transposition: Group III. Pulmonary stenosis and single ventricle. *A*, Roentgenogram demonstrating a normal heart size and diminished pulmonary vascular markings in a 7-year-old boy with marked cyanosis. Venous angiography and right heart catheterization were interpreted as demonstrating transposition of the aorta and pulmonary artery, pulmonic stenosis, and a single ventricle. *B*, Roentgenogram demonstrating heart size unchanged 7 months following anastomosis of the distal end of the right pulmonary artery to the side of the superior vena cava, occluded at right atrium. *C* and *D*, Illustration of the proposed palliative procedure in Group III patients with a single ventricle.

position of origin of the aorta and pulmonary artery may determine the success of the procedure. It is doubtful, with present techniques if 2 functioning ventricles can be constructed from a single chamber where there is no significant septal remnant. Only time and clinical trial will determine if the totally corrective procedure is possible in these patients or if management by palliative procedure is required. In any event, a logical operative approach to all patients in Group III is the performance of vena cava–pulmonary artery shunt initially. This procedure, by the direct delivery of systemic venous blood to the pulmonary artery, bypasses the right side of the heart and the pulmonary stenosis. Because venous pressure, gravity, and respiratory movement are responsible for the flow of blood from the cava into the pulmonary artery, there is no change in the work of the single ventricle as there would be if a systemic artery–pulmonary artery shunt were performed. This will increase the volume of systemic venous blood oxygenated without increasing the work of the heart. If it is determined later that an attempt should be made to correct the transposition and other intracardiac defects, it is not necessary to take down the venous shunt, since its presence will simplify the plan for redirection of the venous blood at the atrial level.

GROUP IV

Six patients with complex anomalies are included in this group. All were examined post mortem. The average duration of life was 7 weeks with a range of 1 to 18 weeks. A patent foramen ovale or atrial defect and a ventricular defect or single ventricle was present in every case. Three patients had a severe preductal coarctation, 2 had an atrioventricular cushion defect and cor biloculare, and 1 showed hypoplasia of the right heart.

Clinical Features.—Cyanosis was present in all patients from birth. Congestive heart failure was prominent from an early age except in one of the patients with deformed atrioventricular valves who was extremely anoxic but showed no overt signs of congestive failure. Diagnostic studies did not reveal the complex nature of these defects except in the case of coarctation.

Operation was performed in 1 patient.

Comment.—It is doubtful if any type of palliative or corrective procedure would be effective in the patients with the hypoplastic right heart or cor biloculare. However, the same principles of management prevail in this group as in the others and if it is possible to provide better intermixing of blood and to protect the pulmonary vasculature, operation may be of value. The patients with coarctation would appear to be salvageable. The logical approach would appear to be resection of the coractation and division of the ductus and then treatment the same as that given patients in Group II.

DISCUSSION

Before operation is undertaken in any patient with transposition, it is imperative that an effort be made, as soon as the anomaly is suspected and before the patient's condition deteriorates, to secure a precise diagnosis of the associated cardiac anomalies. The minimal diagnostic procedure is angiography, preferably by the "ciné" technique and with the dye injected through a catheter directly into either the left atrium or the right or left ventricle. Additional pressure and oxygen data should be obtained, if possible, with the catheter passed into each of the 4 chambers of the heart through defects in the cardiac septum.

Classification of patients with transposition according to the type of associated defect simplifies discussion of the types of surgical procedures that may be used partially or completely to correct the abnormal circulatory dynamics occasioned by the anomalous position of the aorta and pulmonary artery. According to the classification we have established, these cases are divided into 4 groups:

Group I (25 per cent): Transposition with patent foramen ovale
Basic problems:
1. Inadequate intercommunication of systemic and pulmonary circulations (mixing)
2. Pulmonary congestion
Proposed palliative procedures:
1. Atrial septal defect or partial vein transplant
2. Interruption of patent ductus

Group II (45 per cent): Transposition with ventricular septal defect
Basic problems:
1. Pulmonary congestion
2. Inadequate mixing (small and medium-sized defects)
3. Single ventricle
Proposed palliative procedures:
1. Banding (constriction) of pulmonary artery
2. Atrial septal defect or partial vein transplant

Group III (22 per cent): Transposition with pulmonary stenosis and ventricular septal defect
Basic problems:
1. Inadequate pulmonary circulation
2. Single ventricle
Proposed palliative procedures:
1. Vena cava–pulmonary artery shunt

Group IV (8 per cent). Transposition with complex associated deformities
Basic problems and proposed palliative procedures vary with nature of associated deformity

The purpose of the palliative procedures is to allow the patient with transposition to survive beyond infancy so as to permit at a later time the definitive correction of the circulatory alterations caused by the transposed aorta and pulmonary artery. It is doubtful if the patients with single ventricle in Groups II, III, and IV (24 patients, 33 per cent) will be candidates for a corrective procedure. Good palliation should be obtained in these patients with pulmonary stenosis (congenital or surgical) and a vena cava–pulmonary artery shunt.

Complete surgical correction will probably be possible only in Group I, and in Group II in the patients who do not have a single ventricle and who, with the aid of the palliative procedures previously mentioned, can be made to survive beyond infancy. Ideally, corrective surgery should be delayed for several years at least, until the structures are sufficiently large to allow the necessary precise reconstruction, preferably without prosthetic material. If the child should develop signs of distress, corrective surgery would have to be undertaken sooner.

Three types of procedures have been used in an attempt at total correction of transposition: (1) retransposition of the aortic and pulmonary artery roots (the coronary arteries must also be transposed, a major drawback of this procedure); (2) transposition of the systemic and pulmonary veins (partial transposition has had temporary success[23]; Baffes[5] reports that 70 per cent of his patients with partial venous transposition are improved); and (3) transposition of the atrial septum with or without the use of a prosthesis. Albert,[2] Creech,[3] Merendino,[20] Senning,[25] and others have advocated this method which, at the present time, appears to be the most promising. It was the method used by Senning in the single successful case of complete correction—the 9-year-old child with a large atrial defect as the only associated anomaly.

When methods of extracorporeal circulation and profound hypothermia are perfected for use in infants under 6 months of age, it may be possible to perform total correction in many cases heretofore irremediable.

SUMMARY

A series of 72 patients with transposition of the aorta and pulmonary artery have been seen over the past 13 years. Fifty-four have died. Operation has been performed on 32 patients. The clinical features and pathologic findings in these patients have been reviewed in detail.

A classification has been established on the basis of associated cardiac defects. The 2 major problems limiting survival in patients with transposition have been emphasized: (1) inadequate mixing of the pulmonary and systemic circulations, and (2) pulmonary congestion. The evidence suggests that a patent ductus arteriosus may contribute to pulmonary congestion and, except in those with pulmonary stenosis, it seems logical to recommend that this structure be interrupted at the time other palliative procedures are performed.

Palliative (partially corrective) surgical procedures have been proposed as the initial operation for each of 4 groups. It is hoped that a curative procedure will eventually be possible in many patients with transposition who, with the aid of previously performed palliative procedures, have been able to survive infancy and early childhood. It is anticipated, however, that for the time being patients with single ventricle (33 per cent) will be candidates for a palliative procedure only.

REFERENCES

1. Albert, H. M.: Personal communication.
2. Albert, H. M.: Surgical Correction of Transposition of the Great Vessels, S. Forum **5**: 74, 1954.
3. Astley, R., and Parsons, C.: Complete Transposition of the Great Vessels, Brit. Heart J. **14**: 13, 1952.
4. Baffes, T. G.: A New Method for Surgical Correction of Transposition of the Aorta and Pulmonary Artery, Surg. Gynec. & Obst. **102**: 227, 1956.
5. Baffes, T. G.: Personal communication.
6. Blalock, A., and Hanlon, C. R.: Interatrial Septal Defect—Its Experimental Production Under Direct Vision, Surg. Gynec. & Obst. **87**: 183, 1948.
7. Blalock, A., and Hanlon, C. R.: The Surgical Treatment of Complete Transposition of the Aorta and the Pulmonary Artery, Surg. Gynec. & Obst. **90**: 1, 1950.
8. Björk, V. O., and Bouckaert, L.: Complete Transposition of the Aorta and Pulmonary Artery. An Experimental Study of the Surgical Possibilities for Its Treatment, J. Thoracic Surg. **28**: 632, 1954.
9. Creech, O., Jr., Mahaffey, D. E., Sayrgh, S. F., and Sailors, E. L.: Complete Transposition of the Great Vessels, SURGERY **43**: 349, 1958.
10. Fowler, R. E. L., and Ordway, N. K.: Circulatory Dynamics in Complete Transposition of the Great Vessels: Physiologic Considerations With Report of Four Cases, A. M. A. Am. J. Dis. Child. **83**: 414, 1952.
11. Glenn, W. W. L.: Circulatory Bypass of the Right Side of the Heart. IV. Shunt Between Superior Vena Cava and Distal Right Pulmonary Artery—Report of Clinical Application, New England J. Med. **259**: 117, 1958.
12. Glotzer, P., Bloomberg, A. E., and Hurwitt, E. S.: An Experimental Procedure for Correction of Transposition of the Great Vessels, A. M. A. Arch. Surg. **80**: 12, 1960.
13. Heath, D., and Edwards, J. E.: The Pathology of Hypertensive Pulmonary Vascular Disease, Circulation **18**: 533, 1958.
14. Jonsson, B., Ovenfors, C. O., and Senning, Å.: Surgically Corrected Case of Transposition of the Great Vessels, Acta chir. scandinav. **245**: 297, 1959.
15. Kay, E. B., and Cross, F. S.: Surgical Treatment of Transposition of the Great Vessels, SURGERY **38**: 712, 1955.

16. Keith, J. D., Rowe, R. D., and Vlad, P.: Heart Disease in Infancy and Childhood, New York, 1958, The Macmillan Company, chap. 23, p. 502.
17. Lenkei, S. C., Swan, H. J. C., and DuShane, J. W.: Transposition of the Great Vessels With Atrial Septal Defect. A Hemodynamic Study in Two Cases, Circulation 20: 842, 1959.
18. Lev, M.: The Pathological Anatomy of Cardiac Complexes Associated With Transposition of the Arterial Trunks, Lab. Invest. 2: 296, 1953.
19. Lillehei, C. W., and Varco, R. L.: Certain Physiologic, Pathologic and Surgical Features of Complete Transposition of the Great Vessels, SURGERY 34: 376, 1953.
20. Merendino, K. A., Joseph, J. E., Herron, P. W., Thomas, G. I., and Vetto, R. R.: Interatrial Venous Transposition, SURGERY 42: 898, 1957.
21. Merendino, K. A.: Personal communication.
22. Muller, W. H., Jr., and Damman, J. F., Jr.: Treatment of Certain Congenital Malformations of the Heart by the Creation of Pulmonic Stenosis to Reduce Pulmonary Hypertension and Excessive Pulmonary Blood Flow: Preliminary Report, Surg. Gynec. & Obst. 95: 213, 1952.
23. Muller, W. H., Jr.: Personal communication.
24. Mustard, W. T., Chute, A. L., Keith, J. D., Sirek, A., Rowe, R. D., and Vlad, P.: A Surgical Approach to Transposition of the Great Vessels With Extracorporeal Circuit, SURGERY 36: 39, 1954.
25. Senning, A.: Surgical Correction of Transposition of the Great Vessels, SURGERY 45: 966, 1959.
26. Senning, A.: Personal communication.
27. Sirak, H. D., Hosier, D. M., and Clatsworthy, H. W., Jr.: Defects of the Interventricular Septum in Infancy: A Two Stage Approach to Their Surgical Correction, New England J. Med. 260: 147, 1959.
28. Vidone, R. A., and Liebow, A. A.: Anatomical and Functional Studies of the Lung Deprived of Pulmonary Arteries and Veins, With an Application in the Therapy of Transposition of the Great Vessels, Am. J. Path. 33: 539, 1957.

DISCUSSION

DR. DENTON A. COOLEY (Houston, Texas).—Complete transposition of the great vessels remains as one of the most challenging problems in cardiac surgery. Animal experimentation has not been particularly useful since it is just as difficult to produce the lesion experimentally as it is to correct the anomaly clinically. Thus, we are left with a purely clinical approach in which various surgical techniques must be tested on patients. The situation is further complicated by the wide variation in nature of the basic anomaly and the presence of associated defects which may lead to confusion in assessing clinical results.

Recently Dr. John Ochsner has analyzed at autopsy 67 cases of transposition of the great vessels at Texas Children's Hospital. Among the associated defects encountered were ventricular septal defect in 43 (67 per cent), patent ductus arteriosus in 31 (48 per cent), patent foramen ovale in 25 (39 per cent), and a few examples of almost every congenital cardiovascular anomaly were represented. This variety of associated defects may prevent development of a single corrective procedure for application in every patient with transposition.

About 4 years ago when our pump oxygenator was first used, we attempted total correction of complete transposition of the great vessels in 4 patients without a successful results. These patients were treated by a technique of atrial version with a septal patch. We became discouraged and returned to palliative or partially corrective procedures. In our hands the Blalock-Hanlon operation of creating an atrial septal defect by a closed technique has been the most satisfactory and practical. Between 1954 and 1959, 43 patients underwent this operation with a 25 per cent mortality rate. Most of these patients were less than 1 year of age and in critical condition at the time of operation. Indeed, 42 per cent of these patients were less than 3 months of age. As experience has increased with this surgical technique, our results have improved, and only 4 of the last 28 patients died (mortality rate 14 per cent). In general, the clinical improvement in most of these patients has been gratifying with significant improvement in cyanosis. If in the future the mortality rate for the operation of atrial version is reduced to an acceptable figure, these patients who have an artificially created atrial septal defect should be excellent candidates for the corrective procedure. At present, however, we shall continue to use the palliative techniques on our patients.

SURGICAL TREATMENT OF PULMONARY STENOSIS USING EXTRACORPOREAL CIRCULATION

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THE surgical principle of direct relief of obstruction by mechanical means was first applied to the treatment of pulmonary stenosis by Doyen in 1913,[3] and more recently by Sellors[6] and Brock.[2] Since 1948 the method of closed valvotomy for the relief of "pure" pulmonary stenosis has been developed and applied to many patients. Open valvotomy with the use of hypothermia[7] and of extracorporeal circulation[5] has followed.

The purpose of this paper is to review our experience in the treatment of patients with preoperative diagnoses of pulmonic stenosis and to present our perspective on the subject. We have become impressed by the fact that catheter and even angiographic evidence does not always establish the extent of malformations present. As a consequence, any operative approach which does not allow the surgeon adequate exposure of the interior of the heart and ample time and security to cope with unexpected findings will result in incomplete cures. The evolution of unhurried operation under direct vision with the use of cardiopulmonary bypass has brought about a more precise understanding of the pathologic anatomy in the individual patient and, correspondingly, its precise correction.

PRESENT SERIES

There were 55 patients in the series, 53 of whom came to operation with a diagnosis of valvular stenosis, and 2 with congenital infundibular stenosis (Table I). Nine also had atrial septal defects, 1 had associated hypoplasia of the infundibulum, pulmonary artery, and valve ring, and 1 had corrected transposition of the great vessels. Four patients had unsuspected interventricular septal defects. Three of these ventricular septal defects were discovered at operation and the fourth was found as a result of study 1 year after pulmonary valvotomy. The largest of the defects found at operation was 8 mm. in diameter, and none was functionally significant in the presence of right ventricular outflow obstruction. The 55 cases presented do not include those of larger ventricular septal defects carrying a measurable shunt—the so-called acyanotic tetralogies. The problem of how to classify the complex of a small ventricular septal defect in association with pulmonary stenosis has been discussed by McGoon and Kirklin.[5]

Of some interest is the fact that 4 of the 55 were one of twins, and that 2 others had a clear history of past subacute bacterial endocarditis. The patient with outflow tract hypoplasia had multiple congenital abnormalities including a persistent ductus arteriosus which had been divided earlier in childhood.

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TABLE I. CLASSIFICATION OF 55 CASES OF PULMONARY STENOSIS WITH ASSOCIATED ANOMALIES

Valvular stenosis (53)	
Isolated	39
+ Atrial septal defect	9
+ Ventricular septal defect	3
+ Hypoplasia of outflow tract	1
+ Corrected transposition	1
Infundibular stenosis (2)	
Isolated	1
+ Ventricular septal defect	1
	55

OPERATION

The evolution of the surgical management of pulmonary stenosis is reflected in the present series. First, closed transventricular valvotomy by the method described by Brock was used, followed by open transarterial valvotomy as advocated by Swan.[7] Since 1957, all elective operations for pulmonary stenosis have been performed with the use of extracorporeal circulation, and this is our present policy. We have no experience of open valvotomy using inflow occlusion at normal temperatures.[4]

Urgent Relief of Stenosis in Infants and Small Children.—The problem presented by pulmonary stenosis in infants and small children often amounts to a surgical emergency. Practically all of these very young, ill individuals were operated upon before our use of extracorporeal circulation, but it is doubtful that we could safely employ it even now in the small infants. In most instances the closed transventricular operation with moderate hypothermia by surface cooling was employed. In 11 of these small patients, open valvotomy with the use of hypothermia and inflow occlusion was chosen. Five of these, all deeply cyanosed, died at or soon after operation.

Elective Valvotomy.—Eighteen patients had elective operation before the introduction of cardiopulmonary bypass. The average systolic gradient across the pulmonary valve was 98 mm. Hg. One patient died in this group, a 23-year-old woman who had a preoperative right ventricular systolic pressure of 200 mm. Hg. An open transarterial valvotomy was performed with the use of hypothermia, after which right ventricular hypertension persisted. For the first several hours the patient did well, but then developed systemic hypotension and death rapidly followed. It is of great significance that this woman had a severe form of work hypertrophy of the right ventricle which, of course, was unrelieved by the valvotomy.

Postoperative physiologic studies showed that 5 of the 17 survivors had residual right ventricular hypertension. In one of these the pressure fell to normal after a lapse of 2 years. In other words, in 18 patients who had undergone what was considered to be an adequate valvotomy, 1 died from unrelieved acquired infundibular stenosis, and 4 required reoperation with the use of extracorporeal circulation to effect a complete relief of right ventricular hypertension.

Extracorporeal Circulation.—Twenty-nine patients underwent operations performed with the use of cardiopulmonary bypass. The average systolic gradient across the stenosis was 114 mm. Hg. As in the previous group the majority would be classified as having had severe pulmonary stenosis. At operation a high incidence of secondary infundibular stenosis due to muscle hypertrophy was found, occurring in 16 of 28 patients.

There were 3 deaths in the early postoperative period in this group. Early in the series, 2 of the deaths were attributable to the fact that a perfusion technique had been used. The third occurred in a boy aged 4½ years whose preoperative right ventricular systolic pressure was 93 mm. Hg. A transarterial valvotomy was followed by death on the third postoperative day and this was caused by unrelieved secondary infundibular stenosis. Since this experience we have used the transventricular approach in 14 of the last 16 cases, without a death. In these patients the valve stenosis was relieved by inverting it into the

right ventricle where it was usually cut laterally on 2 sides, and the central thickened area was trimmed away. Infundibular muscle, where found to be redundant, was excised (Fig. 1).

Late postoperative study of the first 13 patients whose operations were performed with the use of extracorporeal circulation has shown that 2 adults have persistent right ventricular hypertension. The first had a combined transarterial and transventricular operation which included resection of hypertrophic infundibular muscle. Whereas the preoperative systolic pressure in the right ventricle had been 250 mm. Hg, 1 year after operation it was 95 mm. Hg with a residual systolic gradient of 76 mm. Hg, because of persistent narrowing of the infundibulum. The conclusion drawn is that not enough hypertrophic infundibular muscle was removed.

The second patient underwent a transarterial operation. One year later the right ventricular systolic pressure was found to be virtually unchanged and there was clear evidence of obstruction at the infundibular level. The study also revealed evidence of a small, previously undetected ventricular septal defect. At reoperation the transventricular incision was used and both the ventricular septal defect and the infundibular stenosis were corrected.

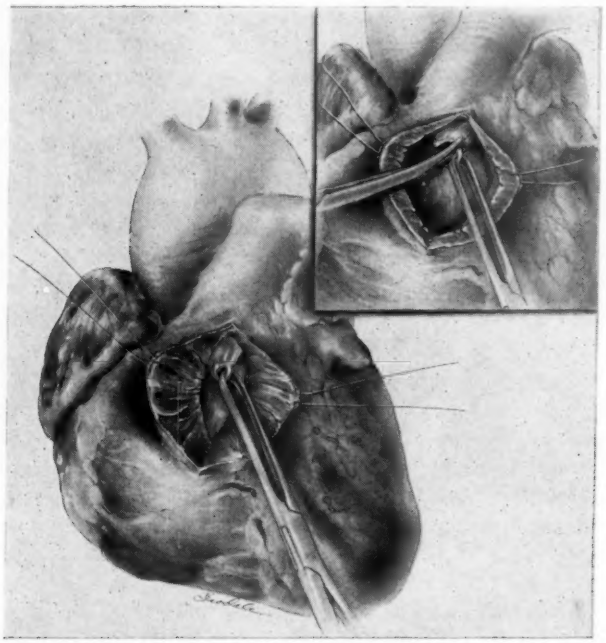

Fig. 1.—Transventricular incision showing the inversion of the stenotic pulmonary valve into the operative field. Note the hypertrophy of the infundibular wall. *Inset*, The excessive infundibular muscle has been excised, and the valve is being opened laterally on 2 sides.

DISCUSSION

The problem presented by the young child with severe pulmonary valve stenosis is a challenging one. With severe right ventricular hypertension, frequently with cyanosis, and often with established right ventricular failure, the situation is correspondingly critical. A swift valvotomy under these circumstances can be lifesaving, instantaneously producing a most remarkable transformation. That these very ill children should carry a high operative risk is not surprising.

Although emergency pulmonary valvotomy by the methods described can be curative, we believe that valvotomy performed on an infant may require further enlargement later in life.

The decision to use cardiopulmonary bypass in the elective treatment of pulmonary stenosis is based on our conviction that complete correction of the deformity in every case is possible only when the operation is carried out under direct vision and without hurry. There was 1 example in the extracorporeal circulation group, referred to previously, of right ventricular outflow tract hypoplasia. Simple relief of the valvular stenosis was not adequate, and complete correction of the obstructive lesion could be achieved only by the insertion of an enlarging gusset. A reinforced autogenous pericardial graft was used, and it is felt that the patient would not have withstood a difficult postoperative course had she not had complete relief of the obstruction in this way.

The advantages of cardiopulmonary bypass can be extended to the correction of other associated anomalies, notably closure of atrial septal defects, without adding appreciably to the risk of the operation.

Secondary Infundibular Stenosis.—This problem has emerged as the single most important factor in the patient's immediate and future welfare. We have previously described this as a form of suicidal heart disease. Although it is true that right ventricular systolic pressures of 100 mm. Hg and over can be sustained after operation, and that in some instances this causes remarkably little disability in the patient while gradual regression of ventricular hypertrophy takes place, it is equally true that such unrelieved obstruction to right ventricular emptying can be lethal soon after operation, and it does not regress as frequently as has been thought. Brock has shown that infundibular hypertrophy may cause obstruction to right ventricular emptying after relief of valvular stenosis, for when there is no obstruction to push against, the infundibular walls contract together more quickly and completely.[1]

It is our belief that there is still another factor acting in some instances. Pulmonary valvotomy may cause valve incompetence which permits regurgitation of blood into the right ventricle during diastole. This regurgitant flow adds to the amount of blood which may be trapped in the right ventricle by the contraction of the infundibular stenosis early in systole (Fig. 2).

It is not always possible to tell from preoperative studies whether work hypertrophy has progressed to a degree that will threaten life after relief of the valvular stenosis. Although older patients with excessively high right ventricular pressures are at greatest risk, it is true that severe infundibular stenosis of the work hypertrophy type can occur in children, and prediction of the altered physiology after valvotomy is uncertain.

Open Transventricular Operation.—We have made increasing use of the transventricular approach, through a short subvalvar ventriculotomy, allowing accurate appraisal of the degree of secondary infundibular stenosis. The valvotomy itself is readily accomplished from below, appropriate muscle excision can be carried out with precision, and an inspection of the interventricular septum can also be made.

In this present series of 29 patients, 16 of those with the diagnosis of "pure" pulmonary valve stenosis were found to have infundibular narrowing

caused by muscle hypertrophy, and in 13 of these resection of muscle was considered necessary to produce an outflow tract of suitable size. The ages of the 13 patients ranged from 5 to 38 and their right ventricular systolic pressures from 90 to 249 mm. Hg. More reliability was placed on inspection of the outflow tract than on pressure determinations, although these were made after completion of the ventriculotomy repair.

For the excision of isolated congenital infundibular stenosis the use of the transventricular approach is clearly essential. The muscular hypertrophy at and below the site of obstruction may be great, and it is usually safer to open the relatively thin-walled infundibular chamber to localize the lumen of the constricted outflow tract from above. Formal excision of the stenosed segment can then be performed with accuracy. Direct access is also an advantage when the precise level of the stenosis is in doubt, as may be the case in high infundibular and the combined varieties of stenosis.

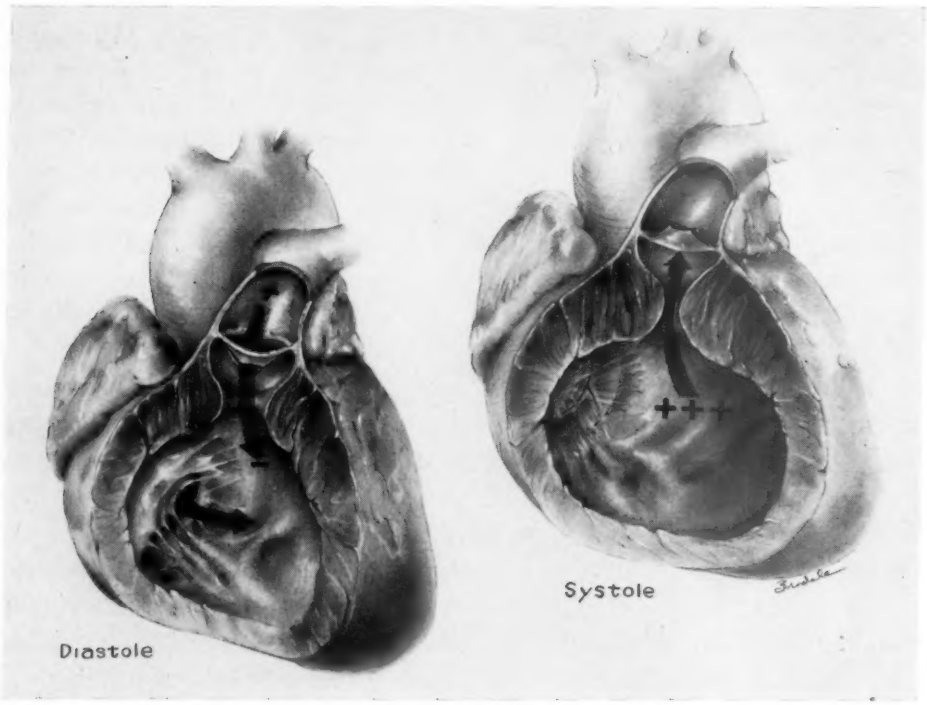

Fig. 2.—Mechanism whereby relief of pulmonary valvular stenosis may exacerbate the effect of secondary infundibular stenosis. During diastole *(left)* the divided valve may allow regurgitation into the ventricle so that during systole *(right)* relatively more blood must pass through the narrow infundibulum.

The transventricular approach is valuable when there is persistent obstruction to right ventricular outflow following an earlier valvotomy. At the second operation, the precise level of obstruction must be found and the obstruction decisively relieved. There have been 5 examples of reoperation in this series. Four were performed with extracorporeal circulation and 1 by a closed method. Of the 4 patients submitted to open operation, 3 were found to have had completely adequate valvotomies. Their persistent ventricular hypertension was

the result of secondary infundibular stenosis. The fourth was found to have both valvular and work hypertrophy stenosis, while the remaining patient has had 2 closed operations for isolated infundibular stenosis and still has a high right ventricular pressure. All those who required a second operation had initial right ventricular pressures of over 94 mm. Hg.

Open inspection of the interventricular septum during repair of pulmonary stenosis has resulted in the unexpected finding of septal defects on 3 occasions. It may be argued that such small ventricular septal defects are compatible with long life and that the only risk is the small one of bacterial endocarditis. We believe, however, that closure of these defects does not add to the risk of the operation, and that the valvotomy may convert a small left-to-right shunt to a large one after the right ventricular hypertension is relieved. It is doubtful that these defects can be found in all instances without a ventriculotomy, for their detection would depend upon the unreliable finding of red blood coming into the pulmonary artery from the heart.

SUMMARY

1. Experience in the surgical relief of pulmonary stenosis is presented with consideration of 55 consecutive operations, the last 29 of which were performed using extracorporeal circulation.

2. Secondary infundibular stenosis was found to be present in more than one-half of the cases repaired electively, and was responsible for 2 postoperative deaths and 5 reoperations.

3. Direct vision of the infundibulum is the only reliable method of evaluating the extent of secondary stenosis, and for this reason we have made increasing use of a transventricular approach in cases diagnosed as "pure" valvular stenosis.

4. Since adoption of this policy there has been no death in 16 cases, and morbidity has been significantly reduced.

REFERENCES

1. Brock, R. C.: Anatomy of Congenital Pulmonary Stenosis, London, 1957, Harper & Brothers.
2. Brock, R. C.: Pulmonary Valvotomy for Relief of Congenital Pulmonary Stenosis; Report of 3 Cases, Brit. M. J. **1**: 1121, 1948.
3. Dumont, J.: Chirurgie des malformations congénitales ou acquises de coeur, Presse méd. **21**: 860, 1913.
4. Lam, C. R., and Taber, R. E.: Simplified Technique for Direct Vision Pulmonary Valvotomy, J. Thoracic Surg. **38**: 309, 1959.
5. McGoon, D. C., and Kirklin, J. W.: Pulmonic Stenosis With Intact Ventricular Septum, Circulation **17**: 180, 1958.
6. Sellors, T. H.: Surgery of Pulmonary Stenosis; Case in Which Pulmonary Valve Was Successfully Divided, Lancet **1**: 988, 1948.
7. Swan, H., and Zeavin, I.: Cessation of Circulation in General Hypothermia; Technics of Intracardiac Surgery Under Direct Vision, Ann. Surg. **139**: 385, 1954.

DISCUSSION

DR. HENRY SWAN (Denver, Colo).—I think Dr. Gerbode and his group are to be congratulated as they continue to explore this difficult problem and not to assume that the techniques currently available are in fact necessarily completely adequate for all the variants found. I would like to ask this question of Dr. Harkins—will you tell us what were

the actual pressures remaining in the 4 patients who had to have reoperation following open valvular operation; what was the order of magnitude of those right ventricular pressures? We have not yet found a pressure postoperatively in patients who have had open valvulotomy, and in individuals in which this was the only anomaly of the heart, which would lead us to desire to reoperate. And as the years have gone by in those patients who had fairly high residual right ventricular pressures following operation these pressures have steadily regressed until they have approached normal. So, therefore, we have had the opinion that this hypertrophy is one of work and is not an actual true anomaly comprising an infundibular stenosis and that once the major obstruction was relieved, it will gradually recede. Apparently, Dr. Gerbode, however, has found at least some in whom it has not receded. On the other hand, though, our experience now includes approximately 63 or 65 patients with isolated valvular stenosis. Since we have not had any operative mortality in that group the open transarterial operative procedure under hypothermia does have the merit of a very low operative risk. We have seen no reason, therefore, to increase the magnitude and the risk by performing infundibular resection.

DR. HARKINS (closing).—The lowest systolic pressure in the 4 patients mentioned in whom we did reoperation was 94 mm. Hg. I think it is interesting that Sir Russell Brock has presented evidence that relief of valvular stenosis may lead to an increase in the physiologic effect of an overgrowth infundibular stenosis because, when there is no valvular stenosis against which to push, the infundibular walls draw closer together. We believe there may be a second factor at play in some cases. A divided pulmonary valve can lead to regurgitation into the right ventricle with the result that there will be more blood accumulated during diastole that has to pass a narrow outflow track during systole. I think this feature of regurgitation considered with Brock's concept of the change in the position of the infundibulum following relief of valvular stenosis accounts for the bad results that we have found.

THE SURGICAL REPAIR OF HIGH PRESSURE VENTRICULAR SEPTAL DEFECT THROUGH THE RIGHT ATRIUM

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(From the Department of Surgery of the University of Southern California School of Medicine, The Los Angeles County Hospital, and the St. Vincent's Hospital)

OF ALL the congenital cardiac malformations seen in infancy and childhood, ventricular septal defect is one of the most common. The presence of symptoms and the establishment of the diagnosis with a predominant left-to-right shunt is usually considered to be indication for surgical repair. The most important prognostic component of the anomaly is the degree of pulmonary hypertension. Our mortality rate in the surgical repair of low to moderate pressure ventricular septal defects has been 2 per cent. However, with pulmonary artery pressures of 70 per cent or more of systemic artery pressures, our mortality rate had been considerably higher, 50 per cent. Because of this high rate, despite what was thought to be good preoperative and postoperative care, we sought other means to decrease mortality. After careful consideration of many factors, we finally reasoned that with the high pulmonary vascular resistance in these patients, the right ventricle with a long incision is not able to propel blood through the lungs adequately. Right-sided heart failure ensued, followed shortly by death. If the ventricular septal defect could be closed without a right ventriculotomy, the chances of survival would probably be greater. The 2 most logical approaches appeared to be repair of the ventricular septal defect through the right atrium or through the left ventricle. Although the latter approach had been successfully used to remove a large left ventricular myxoma by our group,[3] it was felt that the right atrial approach would probably result in the lowest mortality rate. Therefore this approach was used in our last 7 cases of high pressure ventricular septal defects.

SURGICAL APPROACH

A median sternotomy incision is made. Femoral arterial and caval cannulations are performed. The patient is placed on cardiopulmonary bypass. A long incision is made in the right atrium from the atrial appendage down toward the inferior vena cava. The ascending aorta is cross-clamped. Two or 3 minutes are allowed to go by in order to allow sufficient time for the heart to relax before the search for the defect in the ventricular septum. If the heart is stretched during this 2 or 3 minutes before adequate relaxation, complete heart block may occur. This did occur in 1 case even before any sutures were taken in the septum. After 2 or 3 minutes the atrium is opened widely, giving good exposure of the tricuspid valve. By retraction of the septal leaflet of the tricuspid valve cephalad, the

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ventricular septal defect is brought into view. At times it is necessary to divide the septal leaflet of the tricuspid valve to obtain adequate exposure of the defect (Figs. 1 and 2). A Dacron patch is then sewn into the defect. In this series of patients with pulmonary hypertension almost all of the ventricular septal defects were found to have muscular rims. A

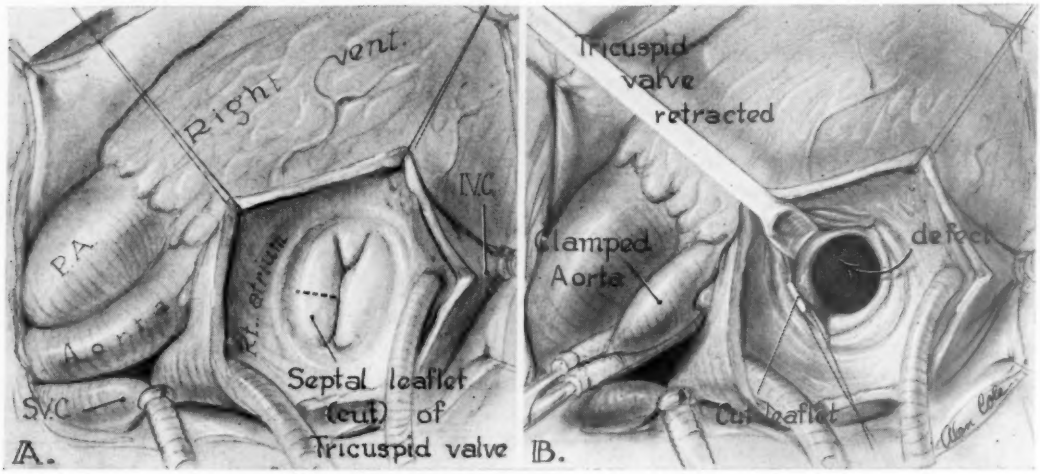

Fig. 1.—The right atrial approach to closure of high pressure ventricular septal defects. In some patients it is necessary to divide the septal leaflet of the tricuspid valve.

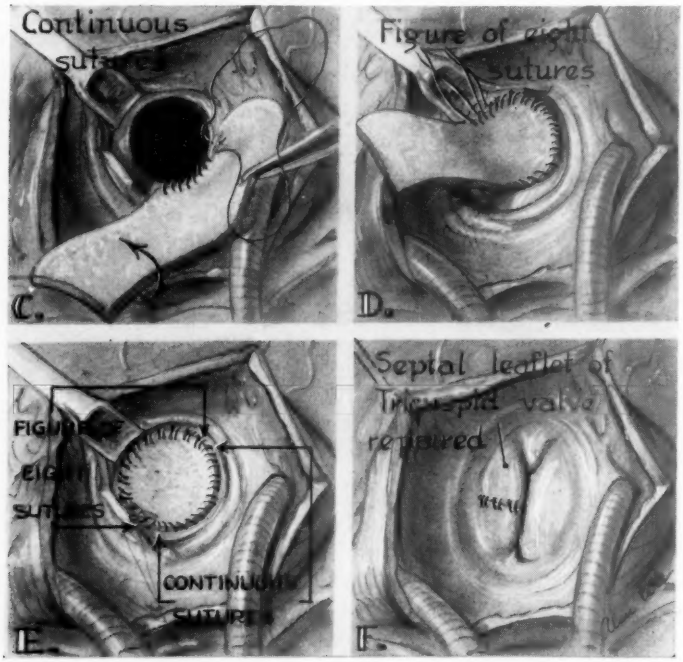

Fig. 2.—Technique of suturing patch in place.

patch was used for closure in most cases. The suturing was started at the angle of the anterior and caudal portion of the defect. There is usually a fibrous portion present at this area. A continuous suture of No. 4-0 silk is run in a posterior direction along the caudal border of the defect. The patch is then sutured to the anterior portion using interrupted figure-of-eight sutures of No. 4-0 silk. These sutures are placed through the right ventricular half of the septum. The suturing is continued in a cephalic direction with figure-of-eight sutures for this part as well as the posterior portion of the defect. After 10 to 12 minutes

of aortic occlusion the aortic clamp is removed and blood is allowed to perfuse the coronary arteries for 3 to 4 minutes. The aorta is again cross-clamped and, after another 2 to 3 minute wait, sewing of the patch is continued. Three or 4 periods of aortic occlusion are usually necessary to complete the suturing of the patch. Prior to placement of the last 2 or 3 sutures in the posterior portion of the defect the aortic clamp is removed and the heart is allowed to recover for 9 or 10 minutes. During this period the heart is defibrillated if ventricular fibrillation is present. The remaining sutures are then placed in the posterior portion of the defect. After closure of the defect with a patch, the incision of the tricuspid valve is sutured with interrupted figure-of-eight sutures of No. 4-0 silk. The right atriotomy is closed with 2 rows of continuous No. 4-0 silk sutures and the patient is taken off cardiopulmonary bypass. The cannulas are then removed, the patient is given Polybrene, the vessels are repaired, and the chest is closed.

RESULTS

Operations were performed in 18 patients with the pulmonary artery systolic pressure above 70 per cent of the systemic artery pressure. In 1 other patient, the pulmonary artery pressure was approximately 60 per cent of the systemic artery pressure.

Right Ventriculotomy.—A right ventriculotomy was performed upon 11 patients. One of these patients was operated on twice with a 10-month interval between the 2 operations. There were 6 deaths in this group.

A 9-year-old girl with a femoral artery pressure of 106/60 mm. Hg and a pulmonary artery pressure of 100/60 with a small left-to-right shunt died approximately 36 hours after re-exploration for bleeding. Re-exploration was performed 12 hours after the original operation. The patient seemed to be doing well but became more cyanotic as time went on and succumbed 48 hours after the original operation. Sections of the lung at autopsy revealed Heath-Edwards[2] classification grade IV.

A 5-year-old girl with a femoral artery pressure of 96/70 mm. Hg, a pulmonary artery pressure of 82/40, and a small left-to-right shunt, and a second 4-year-old girl with a femoral artery pressure of 105/65 and a pulmonary artery pressure of 85/45 with a small left-to-right shunt, died during the operative procedure. Following closure of the defect it was impossible to maintain a good heart beat for any length of time in either patient. Immediately after removal of bypass in both of these patients the central aortic pressure was 110-120 mm. Hg, but within 10 minutes the pressure would gradually fall to 30 or 40 mm. Hg. Cardiopulmonary bypass was reinstituted, but when bypass was removed the pressure would gradually fall to 30-40 mm. Hg. A good beat for any length of time could never be maintained. Sections of the lungs at autopsy revealed Heath-Edwards classification grades IV and V.

A 16-year-old girl with a femoral artery pressure of 100/60 mm. Hg and a pulmonary artery pressure of 70/38 with a moderate left-to-right shunt developed complete heart block during the closure of the defect. The pacemaker was used with the electrodes placed on the right ventricle and subcutaneously near the apex of the heart. Three hours postoperatively the patient was wide awake and appeared to be doing well when suddenly she gasped and lost consciousness. She was taken to the operating room and within 1½ minutes after the onset of the episode the chest was opened and cardiac massage was instituted. Ventricular fibrillation was present. Despite restoration of a good heart beat the patient died 6 hours later. Careful examination of the pacemaker revealed that there was a short circuit which apparently produced the ventricular fibrillation while the heart was being paced. Sections of the lungs at autopsy revealed Heath-Edwards classification grade IV.

A 5-year-old girl with a femoral artery pressure of 100/48 mm. Hg and a pulmonary artery pressure of 92/46 with a small left-to-right shunt appeared to be doing extremely well. However, 24 hours after operation sinus tachycardia with the rate of 170 occurred suddenly and within a few minutes was followed by ventricular fibrillation and death. The

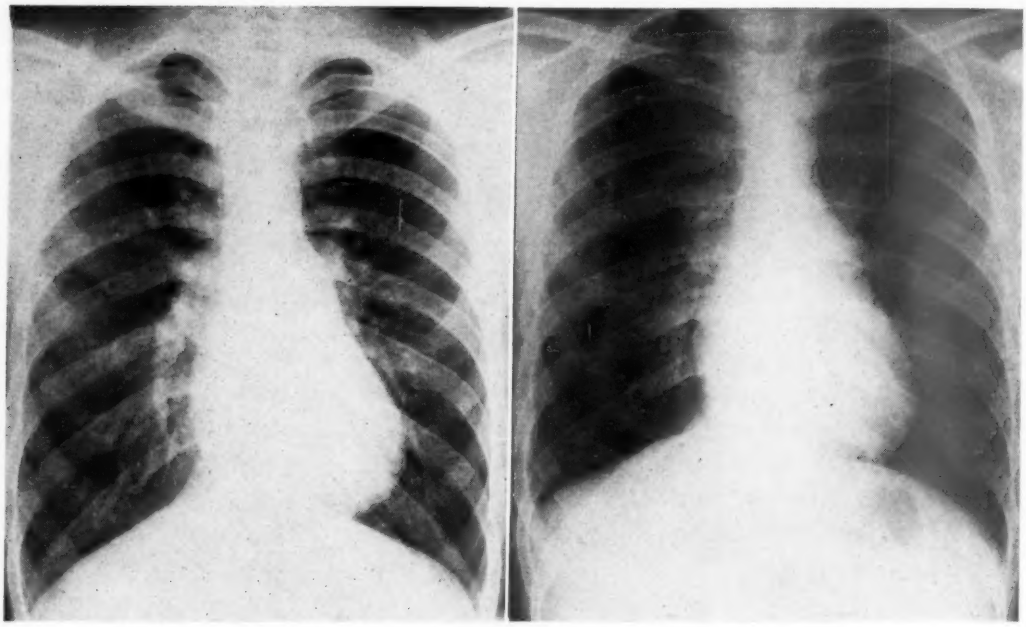

Fig. 3.—Preoperative and postoperative x-rays 5 months later in patient with small left-to-right shunt and equal pulmonary and systemic artery pressures. Defect closed through right atrium.

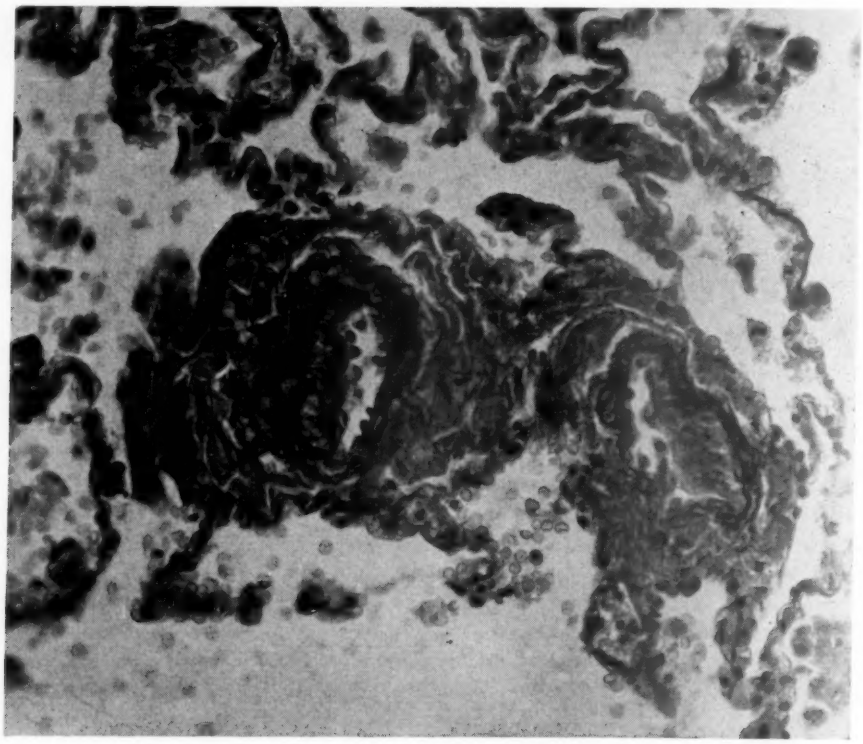

Fig. 4.—The lumen of the small artery is partially occluded by an eccentric proliferation of cellular intimal tissue and fibrosis. (×250, Heath-Edwards grade III.)

chest was opened but a satisfactory beat could not be restored. At autopsy the lungs revealed Heath-Edwards classification grade II.

A 16-year-old boy with a femoral artery pressure of 117/65 mm. Hg, a pulmonary artery pressure of 117/54, and a small left-to-right shunt had been operated upon previously with closure of the defect through the right ventricle. At that time, 1½ years ago, it was felt that some of these muscular defects, even though very large, could be closed with multiple, small, figure-of-eight sutures. This technique was used on 4 patients with high pressure ventricular septal defects or the tetralogy of Fallot. In all 4 patients the defects reopened. This technique was therefore abandoned. The patient with the high pressure ventricular septal defect in whom this technique was used had a stormy postoperative course. A tracheotomy was necessary; however, he did survive. He was reoperated upon 10 months later. The right atrium was opened and an attempt was made to close the defect through it. The defect was inaccessible, however, and a right ventriculotomy had to be performed again. The defect was closed with a Dacron patch. The patient appeared to be doing fairly well for the first 24 hours, but died 4 days after operation with progressive dyspnea despite tracheotomy and positive pressure breathing. At the time of the autopsy there was an area of infarction between the 2 incisions in the right ventricle. The patch was in place and the defect was completely closed. An attempt had been made at the second operation to place the second ventriculotomy in the same area as the first. However, the area of the previous incision could not be determined and consequently the second incision was placed to the right of it. At the time of autopsy this was noted and the area between the 2 incisions was found to be the site of infarction. In this patient, as well as in the previously reported patient who died, an attempt had been made to close the defect through the right atrium. The ventricular septal defect was inaccessible in both of these patients through the atrium, however, and it was therefore closed through the ventricle. Both of these patients died in the postoperative period. Postmortem examination was performed in this last patient who had undergone 2 operations and revealed Heath-Edwards changes grade III.

Right Atriotomy.—There were 8 patients operated upon in this group. Their cardiac catheterization findings were similar to those of the first group. In all patients, except one, the pulmonary artery pressure was 70 per cent or more of the systemic artery pressure.

One patient had a right ventricular systolic pressure of 105 mm. Hg with a systemic systolic pressure of 110 mm. Hg. Because of slight pulmonary subvalvular stenosis, however, the pulmonary artery systolic pressure was 63 mm. Hg. This patient had a corrected transposition of the great vessels with a ventricular septal defect. Although by our criteria of high pressure ventricular septal defects he should not be included in this series, he is being included because it is believed that the atrial approach should not be used in patients with corrected transposition. This belief will be amplified.

A 16-year-old boy with femoral artery pressure of 128/72 mm. Hg, pulmonary artery pressure of 112/48, and a small left-to-right shunt was operated upon. Postoperatively he had a very stormy course. It was necessary to perform a tracheotomy 14 hours after operation and administer constant positive pressure breathing for 7 days. He was also treated with diuretics for edema and hepatomegaly. He gradually responded and now, 8 months postoperatively, he is doing extremely well (Figs. 3 and 4). Sections of lung revealed Heath-Edwards classification grade III.

A similar patient was a small boy 10 years old with femoral artery pressure of 85/55 mm. Hg, pulmonary artery pressure of 76/36, and a small left-to-right shunt. He also required a tracheotomy postoperatively with constant positive pressure breathing but gradually improved and now, 7 months after operation, he is also doing well. X-ray reveals decrease in the pulmonary vascular markings and decrease in the heart size (Figs. 5 and 6). Microscopic studies of the lung revealed Heath-Edwards changes grade III.

A 15-year-old girl had femoral artery pressure of 100/50 mm. Hg and pulmonary artery pressure of 70/30. Cardiac catheterization data were compatible with a ventricular septal defect, atrial septal defect, and patent ductus arteriosus. There was a large left-to-

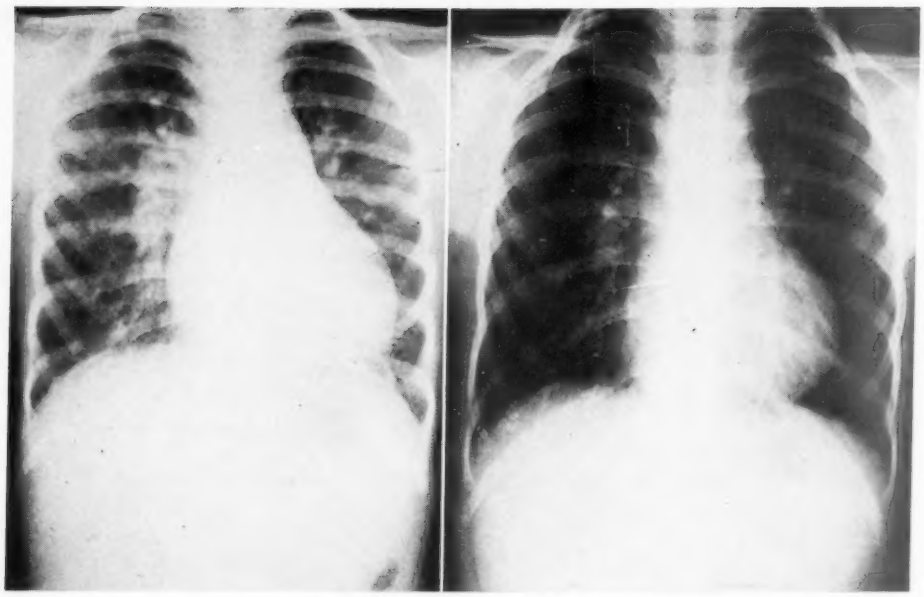

Fig. 5.—Preoperative and postoperative x-rays 6 months later in patient with small left-to-right shunt and equal pulmonary and systemic artery pressures. Defect closed through right atrium.

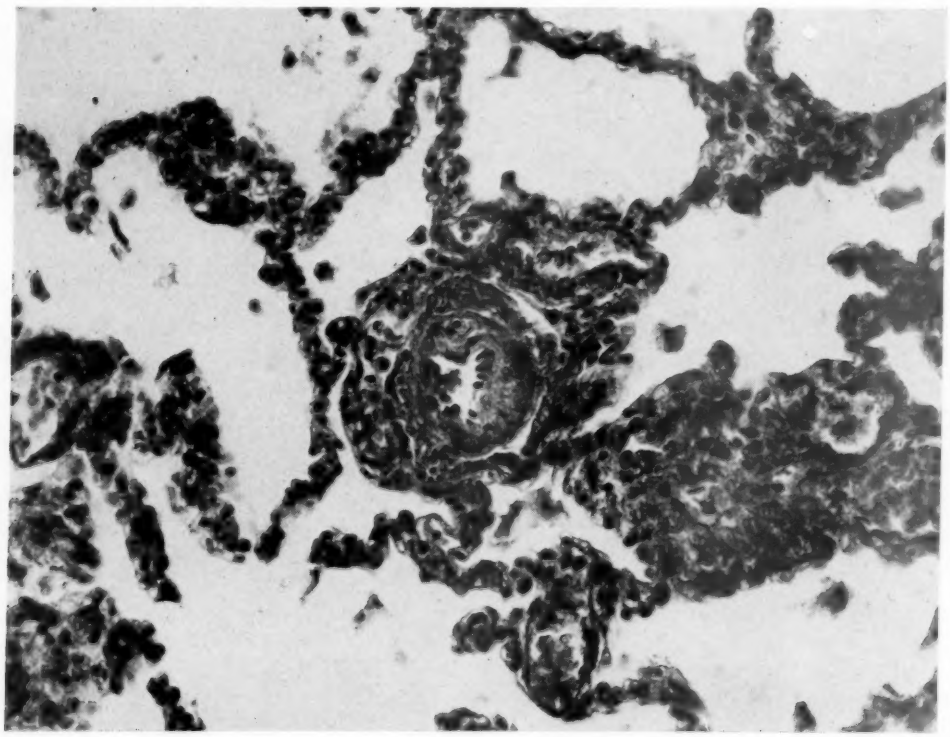

Fig. 6.—The lumen is partially occluded by an eccentric mass of cellular intimal tissue with fairly marked fibrosis. (×250, Heath-Edwards grade III.)

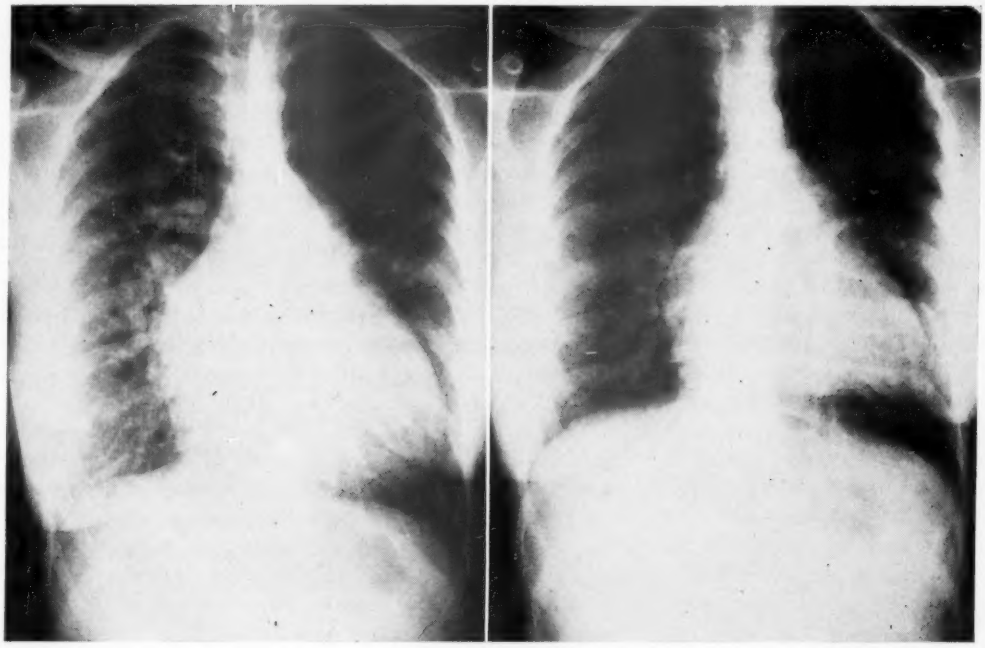

Fig. 7.—Preoperative and postoperative x-rays 3 months later of patient with large left-to-right shunt with a patent ductus arteriosus, atrial septal defect, and ventricular septal defect.

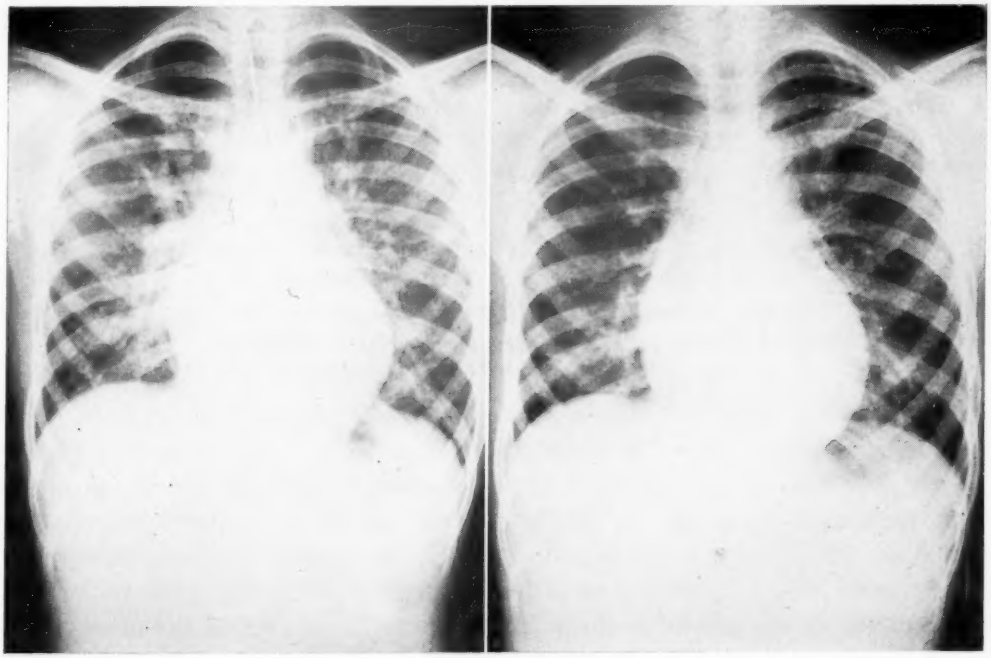

Fig. 8.—Preoperative and postoperative x-rays 10 months later in patient with large left-to-right shunt and equal pulmonary and systemic artery pressure. Almost complete absence of ventricular septum closed with patch through right atrium.

right shunt. All 3 lesions were corrected through the median sternotomy approach, the ductus was ligated, and the atrial and ventricular septal defects were closed through the right atriotomy incision. The postoperative course was benign. The girl did not require a tracheotomy. Her condition is now excellent 4 months after operation (Fig. 7).

A 14-year-old boy had a femoral artery pressure of 120/70 mm. Hg and a pulmonary artery pressure of 120/70 with a moderate left-to-right shunt. At the time of operation, approximately two-thirds of the upper portion of the septum was found to be absent. The defect was 7 by 4 cm. in diameter. It was possible to sew the patch into the ventricular septum through the atrial approach. In this patient the heart was stretched immediately after the aorta was clamped without allowing 2 or 3 minutes for the heart to relax. On release of the aortic clamp complete heart block was noted. No sutures had been taken in the septum at this point. Despite 20 minutes of waiting, with perfusion of the heart and no suturing or manipulation, the complete heart block persisted. The patch was sewn into the defect. Postoperatively a rate of 90 was maintained with a cardiac pacemaker for 9 days. It was then possible to discontinue the use of the pacemaker when normal sinus rhythm returned with the use of Isuprel. The patient has done extremely well (Fig. 8).

The last patient of interest in this group was a 15-year-old boy with corrected transposition of the great vessels, a ventricular septal defect, and minimal subvalvular pulmonary stenosis. The femoral artery pressure was 110/60 mm. Hg, the pulmonary artery pressure was 63/32, and the right ventricular pressure 105/70. There was a moderate left-to-right shunt. This boy is the only patient in the entire series in whom the pulmonary artery pressure was not at least 70 per cent of the systemic pressure. Despite this, the pulmonary vascular changes were very pronounced and graded as Heath-Edwards grade IV. The ventricular septal defect was approximately 3.5 cm. in diameter and located immediately beneath the aorta. It was repaired with a Dacron patch sewn in place through the right atriotomy incision. The tricuspid valve resembled the mitral valve in this patient. It was necessary to cut the leaflet of the tricuspid valve closest to the ventricular septal defect in order to make the repair. The procedure progressed satisfactorily, but a good aortic pressure could not be maintained after bypass. The aortic pressure of 120 mm. Hg rapidly fell to 20 mm. Hg. The patient was placed on cardiopulmonary bypass for 20 minutes and again was taken off. The heart beat well for only a few minutes and the pressure rapidly fell. At this time it was noted that the right ventricle and right atrium were greatly dilated and the pressures in these chambers were very high. The pulmonary artery was collapsed. It was apparent that there was an obstruction to the pulmonary outflow tract. The right atrial incision was reopened and it was noted that the previously cut leaflet of the tricuspid valve was now blocking the exit to the pulmonary artery. Half of this leaflet was excised and the leaflet was then repaired, so that it now no longer blocked the pulmonary outflow area. The patient was taken off cardiopulmonary bypass, the heart now beat well, and a systolic pressure of over 100 mm. Hg was maintained. The duration of cardiopulmonary bypass was 3 hours, the patient was noted to be cyanotic after closure of the median sternotomy incision, and a tracheotomy was performed. He was given positive pressure breathing but he died 8 hours after operation.

DISCUSSION

With the use of the routine right ventriculotomy incision for the closure of ventricular septal defects in patients with pulmonary hypertension, when pulmonary artery pressure was 70 per cent or more of the systemic artery pressure, our mortality rate was 6 deaths in 11 patients operated upon. The total number of operations in these patients was 12 because of the necessity for reoperation in 1 of the patients. This mortality rate may possibly be accounted for in part by the fact that some of the patients were operated upon early in our series of open heart operations before we had learned to avoid heart block, and by the fact that an occasional patient required a second thoracotomy for bleeding. With these causes for death eliminated, however, some patients with

pronounced pulmonary hypertension died despite routine digitalization, intermittent positive pressure breathing and, in some cases, tracheotomy with continuous positive pressure breathing.

It was felt that some of the deaths were caused by right ventricular failure from an incision in a right ventricle that was already overburdened by the increased pulmonary artery resistance. For this reason, it was felt that the results might be improved if the repair of the ventricular septal defect were performed through a right atriotomy. Cooley[1] first mentioned that a ventricular septal defect could be closed through a right atriotomy in a patient with small ventricular septal defects and stated that in some cases it was easier to close the defect by this approach than by the approach through the right ventricle. Lillehei[4] has also mentioned the atrial approach in patients with advanced pulmonary hypertension but also recommends gradual closure with a patch. Sirak and Hosier[5] proposed a two-stage procedure of producing an artificial ductus at the time of repair of the ventricular septal defect. A second operation is performed at a later date to take down the ductus. Their mortality rate with this approach was 3 out of 6 patients.

In our series 7 patients with a high pressure ventricular septal defect and the pulmonary artery pressure at least 70 per cent or higher than the systemic artery pressure were operated upon through the right atrium. In all of these patients the defect was closed and the patients recovered.

In 2 other patients the right atrium was opened in an attempt to close the ventricular septal defect. However, the defect was inaccessible through this approach and therefore the right ventricle was opened and the defect was closed. Both of these patients died in the postoperative period. They are included as 2 of the 6 deaths in the series of 11 patients operated upon through the right ventricle. One other patient is included in this paper although, despite a moderate flow into the pulmonary artery and pronounced pulmonary vascular changes as shown by Heath-Edwards classification grade IV, pulmonary artery pressure was only 60 per cent of the femoral artery pressure. The ventricular septal defect was closed through the right atrial approach. This was the only death in 8 patients with high pressure ventricular septal defects in whom this technique was used. This patient also had a corrected transposition of the great vessels. He died postoperatively after having been on the heart-lung machine for 3 hours. It required this length of time to determine why he failed to maintain a good beat after removal of bypass and to attempt correction. In these patients with corrected transposition of the great vessels, the right atriotomy approach is not indicated. Because of the proximity of the tricuspid valve to the pulmonary artery, the mitral configuration of the tricuspid valve, and the stretching of the tricuspid valve necessary to close the defect, the tricuspid valve is prone to block the pulmonary artery outflow tract.

SUMMARY

The right atrial approach for closure of high pressure ventricular septal defects has been utilized in 7 patients with no deaths. These patients routinely received digitalis preoperatively as well as during the postoperative period, and

intermittent positive pressure breathing. Whenever indicated, tracheotomy and constant positive pressure breathing were also employed. All 7 patients have done well. In an eighth patient with corrected transposition of the great vessels and a pulmonary artery pressure of 60 per cent of systemic pressure, this approach was used. The patient died because of technical difficulties with the tricuspid valve causing pulmonary artery obstruction necessitating a prolonged perfusion to correct it. This approach is probably contraindicated in the correction of ventricular septal defects in patients with corrected transposition of the great vessels.

The mortality rate in the above group has been 13 per cent compared to the 50 per cent rate in those patients in whom the right ventricular approach was used.

ADDENDUM

Three more patients with ventricular septal defects and equal pulmonary artery and aortic pressures have been operated upon with the right atrial approach. All 3 patients did extremely well after closure of the defect without having to undergo a tracheotomy.

REFERENCES

1. Cooley, D. A.: Current Status of Surgical Treatment of Ventricular Septal Defect, Dis. Chest **35**: 1, 1959.
2. Heath, D., and Edwards, J. E.: Pathology of Hypertensive Pulmonary Vascular Disease, Circulation **18**: 533, 1958.
3. Kay, J. H., Anderson, R. M., Meihaus, J., Lewis, R., Magidson, O., Bernstein, S., and Griffith, G. C.: Surgical Removal of an Intracavitary Left Ventricular Myxoma, Circulation **20**: 881-886, 1959.
4. Lillehei, C. W.: Contributions of Open Cardiotomy to the Correction of Congenital and Acquired Cardiac Disease, New England J. Med. **258**: 1044, 1958.
5. Sirak, H. D., and Hosier, D. M.: Creation of a Temporary Artificial Ductus for the Surgical Correction of Ventricular Septal Defects Associated With Severe Pulmonary Hypertension, J. Thoracic Surg. **37**: 1, 1959.

Discussion

(Papers by Gerbode, Ross, Harkins, and Osborn; Kay, Anderson, Tolentino, Dykstra, Shapiro, Meihaus, and Magidson)

DR. C. WALTON LILLEHEI (Minneapolis, Minn.).—I should like to comment briefly upon the surgical treatment of ventricular septal defects associated with severe pulmonary hypertension since we have long been very optimistic about their curability.

The problem of surgical management of patients with high pulmonary pressures is clearly related to the fact that the range of technical error consistent with survival is small and successful results require a high degree of surgical precision in all phases of the management. Thus, any factor such as approach via the right atrium which would widen this narrow margin is of potential value in reducing operative risk.

In our experience the factors associated with increasingly consistent successful results may be subdivided into 4 categories of co-equal importance. First to be emphasized is the significance of a correct and precise anatomic diagnosis preoperatively. For example, a much higher percentage of patients with ventricular septal defects and high pulmonary vascular resistance have associated defects such as patent ductus arteriosus, atrial septal defect, corrected transposition, aortic stenosis, and coarctation of the aorta to mention some of the more common ones. Obviously, it is of crucial importance to the surgeon to know this ahead of time so that the reparative surgery can be expeditiously planned and performed. Second is the importance of a physiologic total body perfusion. Third are the various factors of surgical technique such as use of midline sternotomy, complete repair of the defects, avoidance of the myocardial depression associated with anoxic or potassium arrest, immediate treatment of heart block by use of a myocardial

electrode, and creation of temporary right-to-left shunts where necessary in those with extremely high vascular resistance. Last is the meticulous attention to details of postoperative care including prompt recognition and correction of abnormalities of acid-base equilibrium, blood volume, and ventilation by means of tracheostomy and assisted respiration where necessary.

Early in our experience with the repair of ventricular septal defects it was noted that these patients appeared to have a narrower margin of safety than did the patients with patent ductus and an equivalent degree of pulmonary hypertension treated surgically. This observation served to initiate an experimental study upon the effects of cardiac bypass and ventriculotomy upon right ventricular function (S. Forum, 8: 433-438, 1958). This study clearly confirmed the clinical impression that ventriculotomy impaired ventricular function significantly. Therefore, closure of ventricular defects through an atriotomy was undertaken and in this same report its successful use in a patient with very severe pulmonary hypertension (right ventricle 105/5 and pulmonary artery 95/55 mm. Hg) was described. This particular patient was recatheterized 14 months after operation and was found to have had complete closure of the septal defect with a pulmonary pressure of 34/16 mm. Hg. This initial successful result was distinctly encouraging and during 1957 atriotomy for closure of ventricular defects was utilized in additional patients with severe pulmonary hypertension. However, this added experience indicated to us that the disadvantages outweighed the advantages and this approach now is seldom utilized except for certain unusual types of left ventricular-right atrial shunts and for the ventricular defect component of the complete form of the atrioventricularis communis defects. The disadvantages of the atrial approach are related to inadequate visualization of certain defects resulting in the need to make an additional ventriculotomy, thereby increasing rather than decreasing the amount of cardiac trauma, frequent injuries to the tricuspid valve, increased need for elective arrest as well as longer periods of arrest because of the less adequate exposure, and a greater incidence of postoperative residual shunts. As a result, atriotomy for closure of ventricular defects has had a limited use in recent years in our clinic.

Although ventriculotomy, even when small and placed most favorably, significantly impairs ventricular function, it has been found in our experience to date that the most feasible method to compensate for this occurrence in patients with very high pulmonary vascular resistances is to create a temporary right-to-left shunt which will function in the immediate postoperative interval and will automatically close when no longer needed.

DR. GEORGE H. A. CLOWES, JR. (Cleveland, Ohio).—I wish to comment on both of the last 2 papers, not particularly in relation to technique but to point the moral that both of them show. That is, for survival the heart must be in a condition enabling it to pump the requisite quantity of blood demanded by the body.

Brody and his associates last year observed in their series of patients following cardiotomy that those people who exhibited low cardiac output did poorly, became acidotic, and died.

The question is, how much of the cardiac output is needed postoperatively to do well and to maintain metabolic homeostasis? To establish some sort of base line, my associates, Drs. Del Guercio and Barwinsky, and I have measured the cardiac output in patients who have had thoracotomies for other types of intrathoracic operations. It appears that the normal response is an increase of output to nearly twice the basal level immediately after completion of the operation and a continuation of some degree of elevation during the week thereafter. It has been interesting to observe that our patients who behaved in a similar fashion following cardiotomy did well, whereas those who failed to respond with at least a moderate elevation of cardiac output died. Therefore, it becomes important to recognize and remedy situations which may result later in impaired cardiac function in the immediate postoperative period. Such a situation was illustrated by Dr. Gerbode's patient who died with the infundibular stenosis described by Dr. Harkins. Trauma to the myocardium is another factor which may impair the heart's ability to pump the extra blood required postoperatively. This is what Dr. Anderson avoided by not making an incision in the right ventricle.

HEMODYNAMIC ALTERATIONS IN PATIENTS WITH CEREBRAL ARTERIAL INSUFFICIENCY BEFORE AND AFTER OPERATION

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ATHEROSCLEROTIC occlusive lesions causing cerebral arterial insufficiency may be located extracranially in the internal carotid, vertebral, innominate, common carotid, and subclavian arteries. This fortunate location of the occlusive process and its frequent segmental nature have permitted the development and application of operative procedures designed to restore normal circulation in the main arterial channels. The incidence of these lesions and their susceptibility to reconstructive operation are not known; however, in our experience with arteriographic study of 425 patients with clinical manifestations of cerebral arterial insufficiency, lesions producing varying degrees of occlusion were present extracranially in approximately 40 per cent of the cases. By employment of endarterectomy and bypass graft procedures, circulation was successfully restored in the treatment of 165 lesions occurring in 136 patients.[2] Although the technical and functional results of operation were satisfactory in the majority of these cases, our experience emphasized certain problems associated with the application of this form of therapy. At the present time patients are selected for operation on the basis of arteriographic demonstration of an extracranial lesion. This guide to therapy unfortunately has several limitations. In the first place, secondary lesions in the small, distal intracerebral vessels which may be functionally more significant are not demonstrable by arteriography and, in the second place, the functional significance of lesions in the larger proximal vessels is not evident by this method of study except in the more advanced cases. Moreover, the clinical manifestations of the distal lesions are frequently similar to those associated with lesions in the proximal extracranial arterial segments. For these reasons, it is frequently impossible to localize accurately the level of obstruction on the basis of clinical manifestations. To be sure, it may eventually prove desirable to treat all extracranial lesions for prophylactic reasons. The best functional results, however, will obviously be obtained in the treatment of lesions associated with alterations of cerebral circulation. An important consideration at present is the formulation of clinical criteria by which the functional significance of occlusive lesions may be assessed on the basis of blood flow alterations.

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The second problem associated with arterial reconstruction is concerned with the conduct of operation. The application of either endarterectomy or bypass graft requires a brief period of complete arterial occlusion. Fortunately, collateral circulation is adequate to support normal function during a brief period of occlusion in most cases. However, profound disturbances in function may occur in a small number of patients either because of inadequate collateral circulation or because of bradycardia and hypotension resulting from carotid sinus stimulation. The development of these circulatory changes, which lead to disturbance in cerebral function, is unpredictable. Cardiac depression resulting from reflex stimulation must be detected and treated immediately, and some method of cerebral protection must be available during the period of temporary arterial occlusion. Although several methods, including hypothermia and temporary shunts, have been successfully employed for this purpose, some risk and inconvenience have been associated with their application. For this reason, it is desirable to avoid the use of these procedures whenever possible and, in order to do so, the need for cerebral protection must be predictable before the onset of difficulties. To achieve this objective, certain physiologic data are needed regarding the minimal requirements of cerebral circulation for normal function.

The operative exposure of the involved arterial segments in these cases has provided the opportunity to obtain physiologic studies regarding alterations of circulation produced both by the disease and by its operative correction. These data have been correlated with the clinical features of the disease. The purpose of this report is to present the preliminary observations derived both from this study and from the clinical management of a larger number of cases which appear to have a significant bearing upon these problems.

CLINICAL MATERIAL AND METHODS

Direct Intra-arterial Blood Pressure.—A group of 30 patients with atherosclerotic occlusive lesions, of the internal carotid artery in 22, the vertebral artery in 6, and the great vessels arising from the aortic arch in 2, were selected for this part of the study. The symptom in 3 patients was the presence of an ear noise transmitted from the region of the disease. The clinical manifestations of arterial insufficiency varied in the remaining patients and were graded and tabulated arbitrarily according to the severity of symptoms (Tables I and II). For example, mild, nonspecific cerebral symptoms were regarded as 1 plus, transient paralysis 2 plus, transient paralysis with incomplete recovery 3 plus, and persistent hemiplegia 4 plus. The degree of obstruction resulting from the lesions in these cases was estimated from the lateral arteriograms and at operation by linear measurement. The diameter of the unobstructed lumen in the region of the disease was compared to the diameter of the normal vessel above the region of disease, well beyond the area of poststenotic dilation when present. Arterial occlusion was tabulated as per cent reduction in diameter, and on this basis the obstruction in these cases was estimated to range from 15 to 95 per cent. Diastolic retinal arterial pressure was measured by the technique of ophthalmodynamometry, and in normal controls the retinal artery diastolic pressure was found to be 10 mm. Hg less than that obtained over the axillary artery. Retinal artery pressure was considered reduced when it was less than on the contralateral side and when reduced more than 10 mm. Hg below the axillary pressure. Retinal artery pressure in these cases was tabulated as per cent reduction, which ranged from normal to 30 per cent. The presence of murmurs distal to the region of disease and contralateral lesions were noted and tabulated

TABLE I. CORRELATION OF BLOOD FLOW ALTERATIONS WITH DEGREE OF LUMEN OBSTRUCTION AND THE CAROTID

PATIENT	BEFORE OPERATION			AFTER OPERATION		
	MEAN PRESSURE			MEAN PRESSURE		
	COMMON CAROTID	INTERNAL CAROTID	GRADIENT (MM. HG)	COMMON CAROTID	INTERNAL CAROTID	GRADIENT (MM. HG)
1. E. W. D.	125	125	0	125	125	0
2. G. M.	117	117	0	138	138	0
3. C. G.	125	120	5	150	150	0
4. N. R.	205	205	0	205	205	0
5. L. M. L.	170	162	8	117	111	6
6. M. G. S.	100	100	0	100	100	0
7. W. T. G.	137	135	2		No operation	
8. W. W. T.	175	170	5	142	140	2
9. J. A. G.	165	165	0		No operation	
10. H. Z.	147	145	2	152	152	0
11. S. T. B.	135	110	25	155	165	0
12. C. W. T.	165	135	30	165	145	20
13. J. B.	140	105	35	140	140	0
14. C. G. C.	120	100	20	131	130	1
15. N. T. W.	112	105	7	135	130	5
16. R. S. S.	100	65	35	120	120	0
17. W. G. D.	147	80	67	140	145	0
18. G. M.	129	55	74	155	155	0
19. G. E. E.	137	85	52	125	125	0
20. L. W. D.	120	50	70	120	120	0
21. H. D. L.	175	150	25	140	140	0
22. H. J. M.	165	145	20	165	165	0

with the other clinical data. Finally, all of these patients were followed for periods of up to 12 months to determine the degree of improvement resulting from operation.

Using a multiple channel Waterman or Electronics for Medicine recording apparatus, intra-arterial blood pressure was simultaneously measured immediately proximal and distal to the region of disease, both before and after operation in these cases. Because of the margin of error involved in technique, pressure differences of less than 5 per cent were considered insignificant. Greater pressure differences were considered significant and indicated the presence of pressure gradient across the region of disease. The presence of pressure gradient was assumed to indicate functional arterial obstruction and the restoration of pressure distal to the region of operation equal to that obtained proximally was taken to mean complete operative correction of the obstruction. These data were correlated with the degree of obstruction estimated both at operation and from the arteriograms, the retinal artery pressure, and the clinical manifestations of the disease.

In the application of reconstructive operation in the treatment of occlusive lesions of the internal carotid and vertebral arteries, arterial blood flow was temporarily arrested in the involved segments for periods ranging from 3 seconds to 30 minutes. Intra-arterial blood pressure was simultaneously measured proximal and distal to the isolated segment in 18 of these patients who were submitted to operation under local anesthesia, 17 for occlusion of the internal carotid artery and 1 for that of the vertebral artery (Table III). The level of mean pressure distal to the occlusion was correlated both with the development of neurologic

TABLE II. CORRELATION OF BLOOD FLOW ALTERATIONS WITH DEGREE OF LUMEN OBSTRUCTION AND THE CLIN

PATIENT	BEFORE OPERATION			AFTER OPERATION		
	MEAN PRESSURE		GRADIENT (MM. HG)	MEAN PRESSURE		GRADIENT (MM. HG)
	SUBCLAVIAN	VERTEBRAL		SUBCLAVIAN	VERTEBRAL	
1. S. C.	120	120	0		No operation	
2. G. R.	180	180	0		No operation	
3. T. S.	106	106	0		No operation	
4. F. V.	110	95	15	107	98	9
5. C. E.	85	70	15	100	100	0
6. C. E.	87	71	10	88	85	3

CLINICAL MANIFESTATIONS OF DISEASE IN PATIENTS WITH ATHEROMATOUS LESIONS OF THE INTERNAL ARTERY

PER CENT OBSTRUCTION		OCCLUSION OPPOSITE CAROTID	RETINAL ARTERY PRESSURE (% REDUCTION)	MURMUR	OBSTRUCTIVE PULSE WAVE CHANGES	SEVERITY OF NEUROLOGIC SYMPTOMS	RELIEF OF SYMPTOMS
ARTERIOGRAM	OPERATION						
15	40	0	0	No	0	+	No
30	40	Partial	0	Yes	0	+	No
30	30	0	0	Yes	0	+	No
40	40	0	0	Yes	0	+	No
40	40	—	0	Yes	0	+	—
35	—	0	0	Yes	0	+	—
47	40	0	0	Yes	0	+	No
47	45	0	10	Yes	0	+	Yes
45	—	0	0	Yes	0	+	No
30	—	0	0	Yes	0	+	Yes
60	60	Complete	20	Yes	++	++	Yes
60	60	0	20	Yes	++	Ear noise	Yes
70	75	0	18	Yes	++	+++	Yes
70	70	Partial	20	Yes	++	+++	Yes
80	85	00	10	Yes	+	+++	Yes
85	90	—	30	Yes	+++	+++	Yes
85	90	Complete	20	Yes	+++	++	Yes
85	85	0	20	Yes	+++	Ear noise	Yes
90	100	0	30	Yes	+++	++++	Partial
95	90	0	30	No	+++	++	Yes
55	60	0	—	Yes	+++	Ear noise	Yes
60	60	0	—	No	++	+	Yes

deficits and the presence of contralateral occlusion. The internal carotid artery pressure obtained distal to the region of temporary complete occlusion in these cases represents pressure resulting from collateral blood flow within the cerebrum, since this vessel does not have extracranial branches. The source of this collateral flow was determined during operation for a partially occluding lesion of the internal carotid artery in 1 patient with contralateral complete obstruction of the internal carotid artery. This was accomplished by measuring the intra-arterial pressure in the partially occluded internal carotid artery while clamps were temporarily placed proximally, first across the common carotid artery and then successively across the contralateral external carotid and the homolateral external carotid arteries. Neo-Synephrine (2 c.c. of 0.05 per cent solution) was given intravenously to 2 patients during the period of temporary occlusion to determine the effects of vasopressors upon the blood pressure and collateral flow distal to the region of obstruction. This agent was administered in a third patient, who developed a convulsion immediately upon occlusion of the internal carotid artery, to determine its effectiveness in maintaining adequate cerebral circulation by increasing blood pressure and collateral blood flow during the period of total occlusion. Temporary internal shunts with internal diameters ranging from 2.5 to 3.5 mm. were employed during operation in patients with contralateral occlusion. The effectiveness of these shunts in maintaining blood pressure distal to the occlusion was determined by simultaneous measurements of blood pressure in the segments both proximal and distal to the region of operation.

ICAL MANIFESTATIONS OF DISEASE IN PATIENTS WITH ATHEROMATOUS LESIONS OF THE VERTEBRAL ARTERIES

PER CENT OBSTRUCTION		OCCLUSION OF OPPOSITE VERTEBRAL ARTERY	MURMUR	OBSTRUCTIVE PULSE WAVE CHANGES	SEVERITY OF NEUROLOGIC SYMPTOMS	RELIEF OF SYMPTOMS
ARTERIOGRAM	OPERATION					
30	—	Partial	Yes	0	++	—
30	—	Complete	Yes	0	++	—
33	—	Complete	Yes	0	++	—
65	70	Partial	Yes	+++	++	Yes
85	90	Partial	Yes	+++	+++	Yes
90	90	Partial	Yes	+++	+++	Yes

TABLE III. CORRELATION OF INTERNAL CAROTID ARTERY PRESSURE DURING TEMPORARY PERIODS OF COMPLETE OCCLUSION WITH GROSS NEUROLOGIC REACTION

PATIENT	INTRA-ARTERIAL BLOOD PRESSURE						% REDUCTION IN MEAN PRESSURE	DURATION OF TEMPORARY OCCLUSION (MINUTES)	CONTRALATERAL CAROTID OBSTRUCTION	NEUROLOGIC REACTION
	COMMON CAROTID PROXIMAL TO OCCLUSION			INTERNAL CAROTID DISTAL TO OCCLUSION						
	SYSTOLIC	DIASTOLIC	MEAN	SYSTOLIC	DIASTOLIC	MEAN				
1. N. R.	270	140	205	100	80	90	66	5	0	0
2. C. G.	155	75	115	70	50	60	48	20	0	0
3. E. D.	150	80	115	50	40	45	39	19	0	0
4. C. T.	200	85	142	75	50	62	66	23	0	0
5. R. S.	125	80	102	60	45	52	49	18	0	0
6. G. E.	200	90	145	68	50	59	59	30	0	0
7. J. B.	180	90	135	65	48	56	58	20	0	0
8. N. W.	180	80	130	60	40	50	62	22	0	0
9. W. T.	220	110	165	40	40	40	76	19	0	0
10. S. B.	210	115	162	20	20	20	88	2	Complete	Partial aphasia, weakness of arm
11. H. Z.	180	120	150	95	80	87	42	5	Partial	0
12. C. C.	160	100	130	60	25	42	68	25	Partial	0
13. W. D.	170	110	140	20	5	12	91	3 sec.	Complete	Convulsion
14. F. M.	130	80	105	—	—	42	60	3 sec.	Complete	Convulsion
15. M. L.	200	120	160	80	60	70	66	14	0	0
16. H. M.	180	90	135	80	70	75	45	18	0	0
17. H. L.	200	100	150	90	85	87	42	21	0	0

The arterial pressure measurements were recorded as direct pulsatile pressure, and since the recording device was sensitive to minor changes in pressure, reasonably reliable pulse waves were obtained in these patients both proximal and distal to the lesion. This fortunate circumstance permitted a comparison of the pulse wave characteristics both proximal and distal to the region of disease both before and after operation. Since these patients had hypertension and arteriosclerosis, the pulse waves were frequently abnormal both proximal and distal to the lesion. For purposes of comparison, the proximal pulse wave was considered as the base line wave for that patient and the departure of the distal pulse wave from this base line was graded and tabulated according to the degree of change in order to correlate the evidence of obstruction obtained by this method with that obtained by other methods (Tables I and II). Reduction in amplitude was graded as 1 plus, reduction in amplitude and minor differences in contour 2 plus, "saw-toothed" waves 3 plus, and indistinct flat waves 4 plus.

Carotid Sinus Activity During Operation.—The pulse rate and axillary blood pressure were intermittently recorded by conventional methods during operation in 100 patients submitted to operation for lesions of the carotid arteries. Continuous intra-arterial blood pressure and continuous electrocardiograms were obtained during operation in 13 patients. Atropine sulfate (0.4 mg.) was given immediately before operation to 51 patients, and 62 patients received no atropine before operation. General anesthesia was employed in 16 and local anesthesia (procaine) in 97 patients. Changes in pulse rate greater than 10 per minute or alterations in blood pressure greater than 20 mm. Hg occurring during carotid artery manipulation were considered results of hyperactivity of the carotid sinus, and these changes were correlated with the use of atropine before operation. To determine the most effective method of reversing the depressing effects of carotid sinus reflex activity, 4 types of treatment were employed: (1) atropine sulfate (0.3 mg.) intravenously, (2) dilute Neo-Synephrine solution (0.05 per cent) intravenously, (3) procaine (1 per cent) infiltration of the carotid sheath, and (4) a combination of these methods.

Cerebral Capillary Permeability.—Cerebrospinal fluid pressure was measured in the lumbar region before and after operation in the conventional manner in 7 patients, 3 having had bilateral aortosubclavian bypass, 1 vertebral artery endarterectomy. and 3 endarterectomy of the internal carotid artery. Cephalic vein (antecubital) venous pressure was also obtained in these patients. Cerebrospinal fluid pressure was correlated with venous pressure, cerebral dysfunction, and the degree of swelling in the region of operation. Elevation of cerebrospinal fluid pressure was considered a result of an increase in the permeability of either the arterial or venous capillary bed depending upon the degree of concomitant elevation of venous pressure.

RESULTS

Arterial Pressure Alterations in the Region of Disease.—A significant blood pressure gradient was not measured across the lesion in 14 patients (Patients 1-10 and 15, Table I, and Patients 1-3, Table II) submitted to exploratory operation for lesions associated with lumen obstruction ranging from 15 to 47 per cent, as estimated from the arteriograms and at operation (Figs. 1-3). The clinical manifestations of cerebral dysfunction in these cases were mild and consisted of transient episodic lightheadedness, dizziness, paresthesias, and weakness. Objective evidence of neurological damage was absent, and the retinal artery pressure was normal in the patients with lesions of the internal carotid artery. The atheromatous lesions were located in the internal carotid artery in 11 and in the vertebral artery in 3 patients. A faint systolic murmur was audible in the region of disease in the 11 patients with internal carotid artery disease. A lumen of normal diameter was restored in 9 patients with occlusion of the internal carotid artery by endarterectomy and patch graft

Fig. 1.

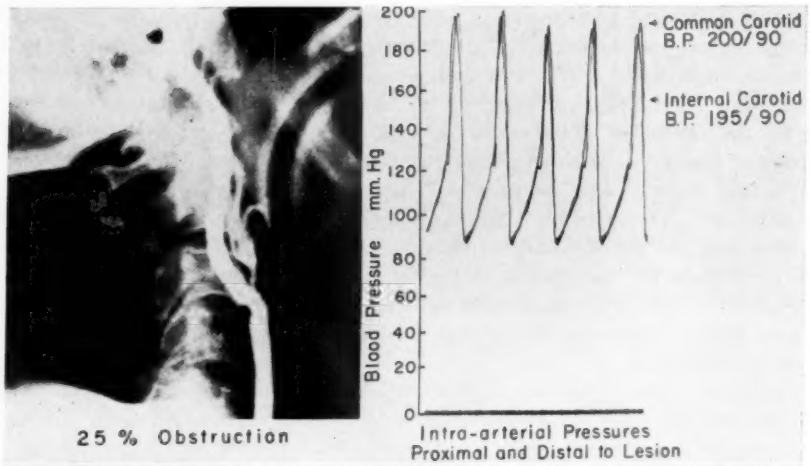

Fig. 2.

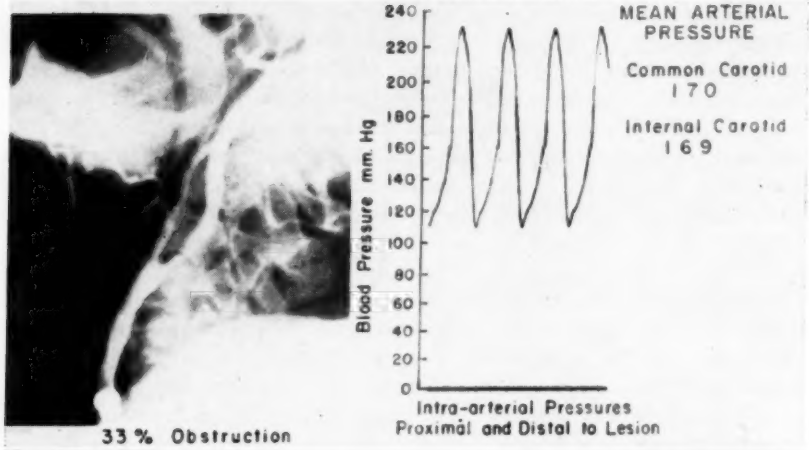

Fig. 3.

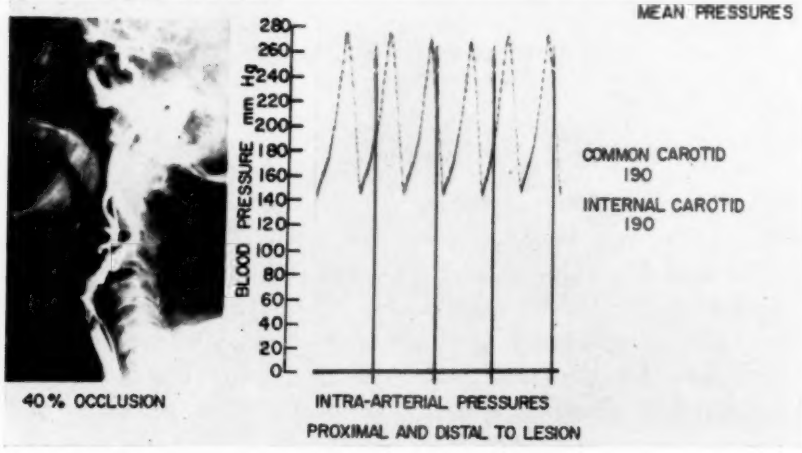

Fig. 1.—Arteriogram and intra-arterial blood pressure recordings demonstrating the absence of a pressure gradient in a patient with 25 per cent reduction in the diameter of the internal carotid artery.

Fig. 2.—Arteriogram and intra-arterial blood pressure recordings demonstrating the absence of a pressure gradient in a patient with 33 per cent reduction in the diameter of the internal carotid artery.

Fig. 3.—Arteriogram and intra-arterial blood pressure recordings demonstrating the absence of a pressure gradient in a patient with 40 per cent reduction in the diameter of the internal carotid artery.

repair as previously described.[2] Reconstructive operation was not employed in 5 patients. The wounds were simply closed in the latter cases after demonstration of the absence of a pressure gradient across the lesion. The pressure recordings were essentially unchanged after operation in those patients submitted to endarterectomy, and the faint systolic murmurs present before operation persisted in 2 cases. The clinical manifestations of disease were not altered by operation. The disease has not progressed in the patients in whom the lesion was not removed, and mild, nonspecific cerebral symptoms present before operation have recurred in most patients in whom the lesion was removed.

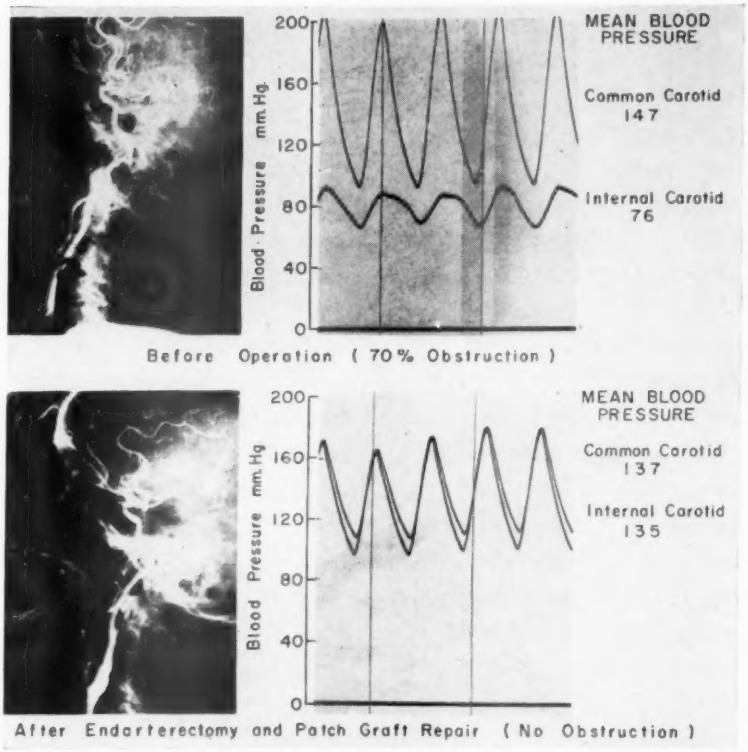

Fig. 4.—Arteriograms and intra-arterial pressure recordings before and after operation showing a pressure gradient and its correction by endarterectomy in a patient with 70 per cent reduction in the diameter of the internal carotid artery.

The lesions in these cases were considered functionally insignificant because of the absence of alterations in blood flow and because removal of the lesions did not significantly change the clinical manifestations of the disease.

A very significant blood pressure gradient was measured across the lesion in the remaining 14 patients (Patients 11-14 and 16-22, Table I, and Patients 4-6, Table II) with occlusion of the internal carotid artery in 11, the vertebral artery in 3, and the great vessels arising from the aortic arch in 2 (Fig. 4). The degree of obstruction estimated from the arteriograms correlated reasonably well with that found at operation and ranged from 50 to 100 per cent. The clinical manifestations of cerebral dysfunction which were appropriately distributed in relationship to the lesion were very specific and obviously of

organic origin. The symptoms, ranging from transient paralysis to persistent hemiplegia, correlated reasonably well with the degree of estimated obstruction, the size of the gradient, and the per cent reduction of retinal artery blood pressure, since the severest symptoms were present in the patients with the greatest objective evidence of obstruction to blood flow. The retinal artery blood pressure was reduced on the involved side in the patients with carotid occlusions in whom this study was obtained, and reasonably loud systolic murmurs were audible in the region of disease in all except 1 patient in whom the lesion was associated with 95 per cent obstruction. Axillary blood pressures and radial pulses were reduced and varied from one side to the other in the 2 patients with occlusion of the innominate and subclavian arteries.

Endarterectomy and patch graft repair were employed in the treatment of patients with occlusion of the internal carotid and vertebral arteries, and the region of occlusion was bypassed in the 2 patients with lesions of the great vessels arising from the aortic arch.[2] Simultaneous intra-arterial pressure measurements both proximal and distal to the region of disease were made immediately after operation. Reduction of the gradient present before operation was considered evidence of success in restoration of reasonably normal blood flow (Fig. 4). This objective was achieved in all patients except one submitted to endarterectomy for occlusion of the internal carotid artery. The intra-arterial pressure measurements obtained immediately after operation in this case showed the gradient to be unchanged (Fig. 5). Endarterectomy had been performed through a longitudinal incision which had been closed by simple suture opposing the wound edges. An arteriogram performed at operation revealed that severe luminal constriction resulting from arterial wound closure was present in the region of operation. As a result of these findings, the arterial incision was reopened and then closed by insertion of a patch graft sutured circumferentially to the edges of the arterial wound. The pressure measurements obtained after this procedure were equal both proximal and distal to the region of operation, and an arteriogram performed 10 days after operation showed normal arterial channels.

These patients with obstruction producing pressure gradients have been followed for 6 to 12 months after reconstructive operation, and improvement has occurred in the clinical manifestations of the disease in all instances. Patients who had had frequent episodic attacks of paralysis have remained asymptomatic since operation, and hemiplegia was considerably improved in the patient with this severe defect. Persistent incomplete paralysis was significantly improved or relieved in the 8 patients with this symptom. The clinical significance of the lesion in one of the latter patients was evident by the fact that partial paralysis which was relieved by vertebral artery endarterectomy recurred 6 months after operation because of recurrent occlusion in the region of operation. As a result of the close correlation between appropriate neurological dysfunction and occlusive lesions associated with clinical and laboratory evidence of reduction in blood flow before operation, and because of

the correlation between the relief of symptoms and restoration of reasonably normal blood flow by operation, the lesions in these cases were considered functionally significant.

Effects of Temporary Occlusion Required for Operation on Distal Arterial Pressure.—The mean arterial blood pressure measured in the internal carotid artery distal to the region of complete occlusion during operation in 17 patients ranged from 12 to 90 mm. Hg depending upon the systemic blood pressure and the presence of contralateral occlusion (Table III). The highest blood pressure

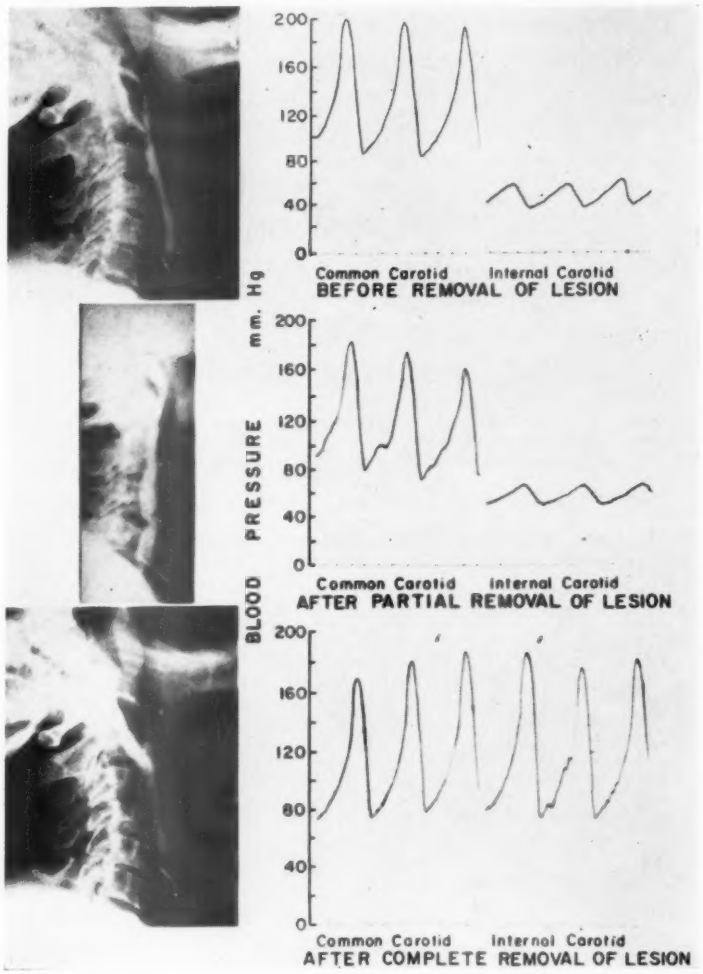

Fig. 5.—Arteriogram and intra-arterial blood pressure recordings made before, during, and after operation showing the usefulness of blood pressure measurements in appraising completeness of operation.

levels were obtained in hypertensive patients with normal contralateral internal carotid arteries, and the lowest pressures were measured in the 3 patients with complete occlusion of the contralateral internal carotid arteries. The period of occlusion in these cases ranged from 3 seconds to 30 minutes. Gross neurological deficit occurred in only 3 patients. Partial aphasia and weakness of the arm

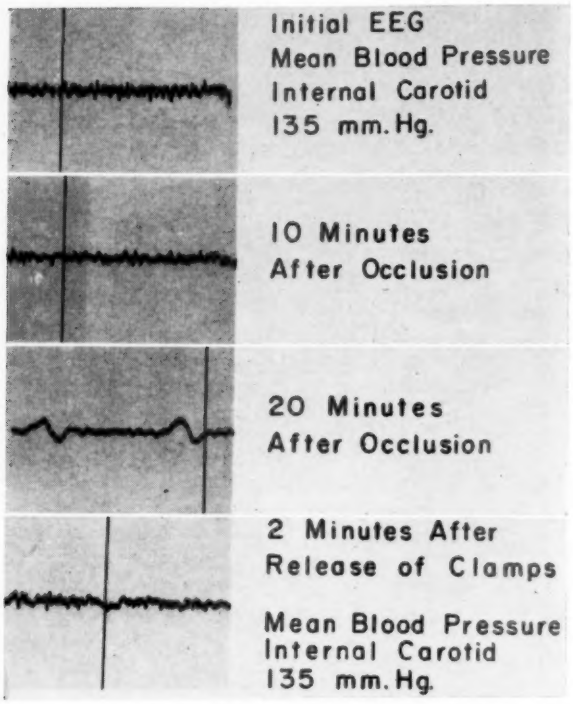

Fig. 6.—Segments of a continuous electroencephalogram showing diminution in amplitude of fast waves and the appearance of slow waves after 20 minutes of arterial occlusion indicating inadequate cerebral circulation with an internal carotid artery mean pressure of 40 mm. Hg.

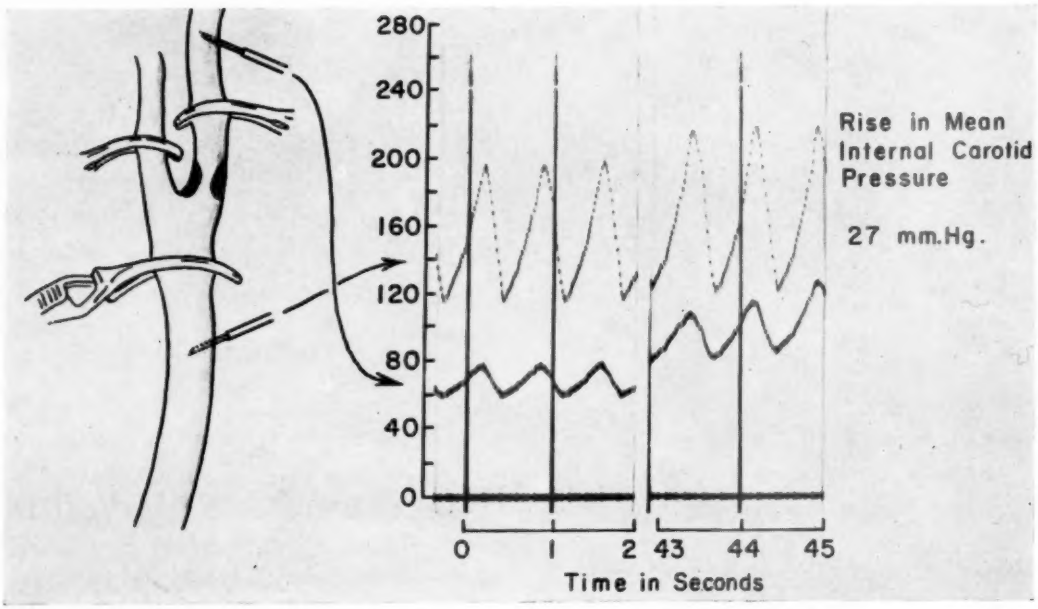

Fig. 7.—Diagrams and intra-arterial blood pressure recordings made during the administration of Neo-Synephrine solution showing rise in internal carotid artery mean pressure distal to segments of vessel isolated for operation.

developed after 2 minutes of occlusion in 1, and a generalized convulsion occurred in the other 2 patients 3 seconds after complete occlusion. The contralateral internal carotid artery was completely occluded in these patients. The mean pressure in the internal carotid artery distal to the region of occlusion was 20 mm. Hg in the first, 12 mm. Hg in the second, and 42 mm. Hg in the third. Although gross neurological dysfunction did not occur during the period of occlusion in the remaining 14 patients with mean internal carotid artery pressures ranging from 40 to 90 mm. Hg (despite partial occlusion of the contralateral internal carotid artery in 2 patients), there was electroencephalographic evidence of ischemia in 1 patient with a mean pressure of 40 mm. Hg (Fig. 6). These signs were evident by diminution in amplitude of the patient's normal brain waves present before occlusion and by the appearance of slow waves after 20 minutes of complete occlusion, during which time the internal carotid artery mean pressure remained constant. These electroencephalographic changes disappeared within 2 minutes after restoration of normal blood flow. Intra-arterial blood pressure was measured in the vertebral artery distal to the segment isolated for operation in 1 case. This patient had complete occlusion of the contralateral vertebral artery. The mean pressure measured distal to the region of operation was 40 mm. Hg and severe neurological disturbance consisting of stupor and weakness on the right side occurred in this patient 30 seconds after complete occlusion. The patient recovered immediately after the clamps were removed.

Neo-Synephrine solution was administered intravenously to 2 patients in whom pressure measurements were obtained during the period of complete internal carotid artery occlusion. This agent produced rapid significant rises in mean pressure distal to the region of operation, 20 mm. Hg in one and 27 mm. Hg in the other (Fig. 7). Neo-Synephrine solution was given to a third patient who had developed a convulsion 3 seconds after temporary occlusion of the internal carotid artery to test its effectiveness in maintaining normal cerebral function. Despite the fact that the mean brachial arterial blood pressure was raised from 105 to 175 mm. Hg, the patient developed a second convulsion 45 seconds after occlusion indicating the ineffectiveness of this agent in the support of cerebral circulation in this case. Temporary internal shunts with diameters ranging from 2.5 to 3.5 mm., employed during the period of complete occlusion, maintained pressures distal to the region of operation essentially equal to that measured in the common carotid artery proximal to the occlusion (Fig. 8). Moreover, cerebral dysfunction occurring during brief periods of complete occlusion were prevented by the use of these shunts, although cerebral blood flow was dependent upon their function for periods of up to 30 minutes.

As previously indicated, the intra-arterial pressure measured in the internal carotid artery beyond the region of temporary occlusion represents intracerebral collateral circulation and the various extracranial components contributing to this collateral flow were measured in 1 patient with complete occlusion of the left internal carotid artery and 80 per cent occlusion of the right internal carotid artery. The common, external, and internal carotid arteries were exposed on both sides. The initial mean pressure in the right common carotid

artery was 150 mm. Hg and the initial mean pressure in the right internal carotid artery was 87 mm. Hg. The subsequent mean pressures obtained in the right internal carotid artery distal to the lesion were 67 mm. Hg with the right common carotid artery clamped, 47 mm. Hg when clamps were placed across the right and left external carotid arteries, 12 mm. Hg after clamping of the left and right external carotid arteries and the right common carotid artery, thus eliminating all direct and collateral carotid artery blood flow to the brain. These blood pressure measurements indicated that the principal routes of collateral circulation were through the posterior communicating artery from the

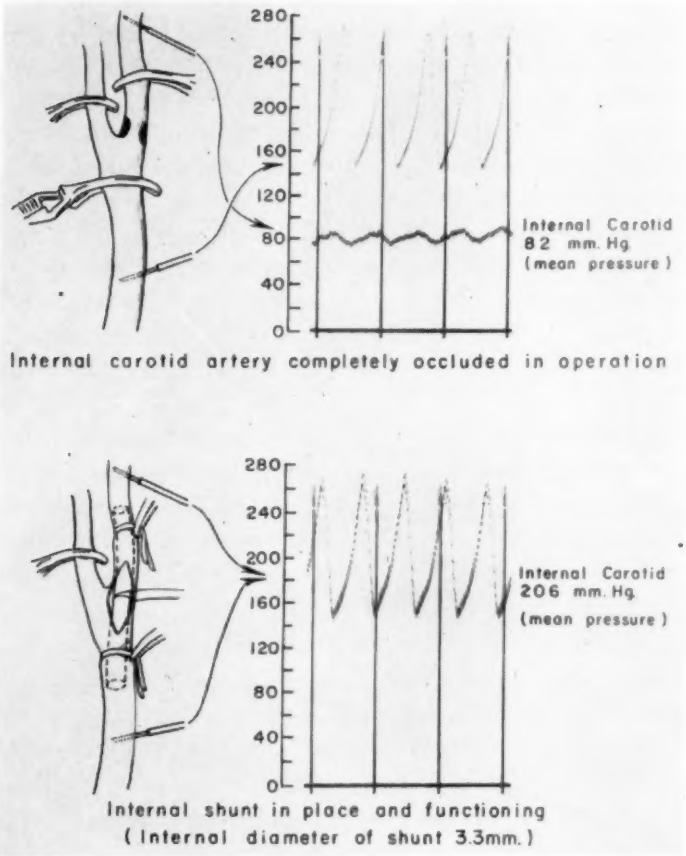

Fig. 8.—Diagram and intra-arterial blood pressure recordings made with temporary internal shunt in place and functioning showing equal pressure proximal and distal to region of operation.

vertebral-basilar system and through the ophthalmic arteries from both external carotid arteries.

Pulse Wave Characteristics.—The characteristics of the pulse waves obtained in the vessels proximal to the lesion were frequently normal and remained unchanged after operation. The amplitude of the wave at this level corresponded to the axillary systolic pressure, and the contour of the waves usually contained sharp peaks and dicrotic notches. The character of the pulse waves obtained in the arterial segments distal to the lesions varied according to the degree of

obstruction. The distal pulse waves were similar to those obtained proximally in patients in whom the lesion was not associated with a pressure gradient (Figs. 1-3). In this series of cases alteration in pulse wave characteristics occurred only in patients with lesions consuming 50 per cent or more of the diameter of the artery and causing a pressure gradient in the region of disease (Fig. 4). The pulse wave changes obtained distal to the lesion in the latter cases varied with the magnitude of the blood pressure change produced by the lesion (Tables I and II). Small gradients were associated only with reduction in amplitude of the pulse wave, the contour of the wave remaining unchanged. As the degree of obstruction increased, the amplitude of the pulse wave decreased, and corresponding changes occurred in the contour of the wave. With moderate obstruction the dicrotic notches and peaks disappeared leaving low, rounded waves of the "saw-tooth" type. These changes in pulse wave characteristics were abolished by successful restoration of circulation. The amplitude and contour of the distal pulse wave became similar to or identical with that obtained proximally in patients in whom the pressure gradient was eliminated by operation (Fig. 4).

Carotid Sinus Activity During Operation.—Significant changes in blood pressure occurred during operation in the majority of cases. The type of change varied according to the type of operative manipulation performed at the time of change. Hypotension occurred during dissection in the region of the carotid sinus in 45 patients (40 per cent), on clamping of the common carotid artery in 14 patients (13 per cent), and immediately after release of the clamps at the end of operation in 71 patients (63 per cent). Significant elevations of blood pressure occurred during the dissection in 41 patients (37 per cent), on clamping of the vessels in 56 patients (50 per cent), and upon release of the clamps in 10 patients (9 per cent). Significant changes in pulse rate occurred in 50 patients (40 per cent). The pulse rate increased in 20 patients (17 per cent) and bradycardia associated with hypotension occurred in 30 patients (26 per cent). The latter form of cardiac depression occurred most frequently during manipulation of the carotid arteries. These changes in pulse rate and blood pressure were not affected by the use of atropine sulfate given before operation. However, the incidence of change varied tremendously with the type of anesthesia employed. Some type of change, usually bradycardia and hypotension, occurred in 13 of the 16 patients submitted to operation under general anesthesia. Local anesthesia, including procaine injection of the carotid sheath, was considerably less frequently associated with changes in blood pressure and pulse rate.

The elevations in blood pressure and pulse rate occurring during operation in these cases were considered inconsequential and were therefore not specifically treated. Since bradycardia and hypotension were associated with further reduction in cerebral circulation, immediate attempts were made to reverse these changes when they appeared. Atropine sulfate (0.3 mg.) given intravenously usually reversed the bradycardia after several minutes, and after a similarly long period of time both bradycardia and hypotension could be reversed by

thoroughly injecting the carotid sheath with procaine solution. Hypotension was immediately reversible in all cases with Neo-Synephrine (0.05 per cent solution) given intravenously. This agent, however, appeared to have considerably less effect upon pulse rate.

Cerebral Capillary Permeability.—Cerebrospinal fluid pressure and venous pressure measured 2 and 4 days after operation were normal in 4 patients, 3 of whom had undergone unilateral internal carotid artery endarterectomy and 1 of whom had undergone unilateral vertebral artery endarterectomy. Significant elevations in these measurements occurred in the 3 patients submitted to bilateral aortosubclavian artery bypass graft (Fig. 9). The duration of elevation ranged from the second to the thirteenth day after operation with the highest elevation occurring on the fourth day. These patients were mentally

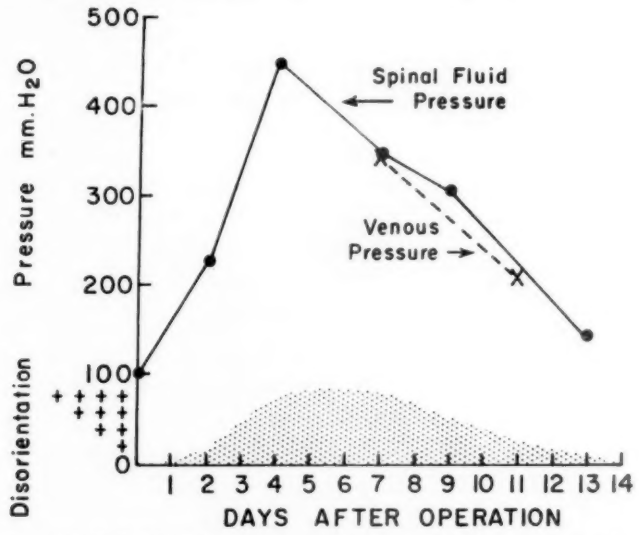

Fig. 9.—Cerebrospinal fluid and venous pressure correlated with mental status of patient submitted to bilateral aortosubclavian bypass.

clear before operation and for the first 36 to 48 hours after operation. Mental confusion and disorientation appeared during the second postoperative day, reaching a peak about the fourth to the sixth day, gradually subsiding by the fourteenth day after operation, corresponding reasonably well with the degree of cerebral edema. The elevations of cerebrospinal fluid pressure were associated with elevations in venous pressure resulting from the nature of the operative procedure. Combined cervicothoracic incisions splitting the sternum in the midline were employed in these cases to insert the bifurcation Dacron aortic graft, which extended from the ascending aorta in the chest to both subclavian arteries in the supraclavicular regions in the base of the neck. Severe swelling occurred in the neck and upper mediastinum after operation. For this reason and because of the presence of a large graft in the thoracic inlet, obstruction occurred in the airway and the major veins draining the head, neck, and upper extremities. Because of the associated venous obstruction, cerebral edema in these cases was considered caused by increased capillary permeability resulting from outflow obstruction located in the neck and thorax.

DISCUSSION

Direct intra-arterial blood pressure measurement was selected as the principal method of studying alterations in cerebral circulation in these patients because of its simplicity. To be sure, the blood pressure measurement does not reflect volume flow. However, changes in blood pressure may be assumed to be associated with changes in blood flow as applied in these cases. For example, the demonstration of a pressure gradient in the region of disease can be accepted as reasonable evidence of obstruction and decreased blood flow not only in the region of disease but in the cerebrum as well. Cerebral blood flow is related directly with blood pressure because of the relative low constant peripheral resistance in the intracerebral vessels, and measurements of pressure in the cervical segments of cerebral circulation have been shown to be comparable to simultaneous measurements made in the intracerebral vessels both before and after temporary occlusion.[1, 3] For these reasons changes in intra-arterial pressure obtained in the neck in patients with extracranial occlusions producing cerebral arterial insufficiency are considered valid indication of changes in blood flow in the region of disease as well as in the distal intracerebral vessels. These studies, because of the limited number and type of observations, do not permit final conclusions which would rigidly govern the application of reconstruction operation in the treatment of cerebral arterial insufficiency. However, in view of the general lack of experience in this form of therapy, those physiologic and clinical observations which appear to have clinical significance deserve some consideration at this time for their value as a guide both to therapy and to further investigation. For convenience these are considered separately.

Selection of Patients for Operation.—Patients with extracranial lesions are selected for operation on the basis of evidence indicating blood flow alterations distal to the lesion. The symptoms in these patients are appropriately distributed in relationship to the location of the lesion, and alteration of blood flow may be suggested clinically by certain physical findings depending upon the location of the lesion. Significant occlusion of the great vessels arising from the aortic arch is usually associated with blood pressure and pulse changes in the neck and upper extremities. Significant internal carotid and common carotid artery occlusions may be suspected from reduced retinal artery pressure. Although the presence of murmurs in the base of the neck in patients with basilar artery insufficiency may suggest the presence of vertebral artery lesions, this physical sign does not necessarily indicate changes in blood flow. The pressure studies performed in this series of patients indicate that the presence of murmurs does not reflect the functional significance of a lesion. For example, murmurs were present in some patients with lesions which were not associated with pressure gradients, and murmurs were absent in others with lesions causing severe reduction in blood flow. Moreover, experience indicates that retinal artery pressure may be reasonably normal in patients with lesions of the internal carotid arteries which produce significant obstruction, despite the good correlation between reductions in retinal artery pressure and the presence of a blood pressure gradient in the small number of patients in whom intra-arterial

blood pressure measurements were made. For these reasons blood flow alterations are frequently not evident on clinical grounds, and the functional significance of a lesion must be estimated from the degree of lumen obstruction visualized arteriographically. Estimates of obstruction obtained from arteriograms made in a single plane may be misleading. However, in this small group of cases studied with intra-arterial pressure measurements, the estimate of obstruction made from the arteriograms correlated very well with the degree of obstruction found at operation. Despite the limitations of this method of estimating obstruction, the best arteriographic indication of functional obstruction at present is 50 per cent reduction in lumen diameter because pressure gradients were demonstrated in all patients with more than 50 per cent obstruction. This finding correlates with the work of Mann and associates,[4] who showed that little reduction in blood flow occurred in the carotid arteries of dogs with less than 50 per cent reduction in the inside diameter, whereas blood flow dropped precipitously with increasing reduction in inside diameter.

Cerebral Protection During the Period of Occlusion Required for Operation.—Collateral circulation is usually adequate to support normal cerebral function during operation in most patients. However, because of the limitation imposed on collateral blood flow by occlusive lesions occurring in the vessels depended upon for collateral flow, severe neurological defects occasionally develop during the period of complete occlusion required for operation. The possibility of this difficulty may be predicted to some extent on the basis of the presence of contralateral occlusion, the development of electroencephalographic and neurological changes upon arterial compression before operation, or upon temporary occlusion at operation. In the small series of cases in which intra-arterial blood pressure measurements were made, collateral blood flow maintaining a distal mean pressure over 42 mm. Hg was sufficient to maintain normal function. Additional experience may prove this method helpful in the determination of minimal blood flow required during the period of operation.

The increase in collateral blood flow provided by the administration of Neo-Synephrine solution in 1 case was inadequate to support normal function as evidenced by the development of a seizure 45 seconds after complete occlusion. For this reason, the usefulness of this agent was considered limited to the control of hypotension occurring during operation. The use of temporary internal shunts is considered the preferred method of maintaining cerebral blood flow in patients with limited collateral circulation because of its effectiveness and because of the simplicity of its application.

Carotid Sinus Activity.—Maintenance of the patient's normal preoperative blood pressure during operation is essential because reduction in systemic arterial blood pressure, particularly during the period of temporary arterial occlusion, may result in severe reduction in cerebral blood flow. Moreover, since these patients frequently have associated occlusive lesions in the vessels supplying blood to other organs such as the heart and kidneys, severe diffuse ischemic damage may result from reduced flow associated with systemic hypotension. The principal factor leading to hypotension during operation in patients with

occlusive disease of the carotid arteries is cardiac depression caused by carotid sinus stimulation. The control of hypotension is concerned primarily with the prevention and treatment of carotid sinus reflex activity. The occurrence of bradycardia and hypotension during carotid artery manipulation is unpredictable. Atropine sulfate given in relatively large doses before operation does not appear to have a significant effect in their prevention. However, these changes do occur less frequently in patients submitted to operation under local anesthesia (procaine) than with general anesthesia, indicating that local anesthetization of the carotid sinus may be the most effective method of preventing carotid sinus activity during operation. The most important consideration is the fact that hypotension occurring in these cases is immediately reversible by the use of intravenous Neo-Synephrine solution and this agent should be available for immediate administration in these cases.

Increased Intracerebral Capillary Permeability.—Although it is suspected that cerebral edema may occur after operation as a result of increased blood flow and increased intracerebral capillary permeability in patients with the more advanced forms of ischemic brain damage, patients of this type have not been studied. Cerebral edema, evident by mental obtundity and increased cerebrospinal fluid pressure, was detected in 3 patients submitted to bilateral aorto-subclavian artery bypass. The airway obstruction and mental derangement in these patients considerably complicated the postoperative management and emphasized the need for the prevention of these disturbances. Since these complications resulted from obstruction in the neck and mediastinum primarily from the use of large grafts, this problem has been alleviated by the employment of smaller grafts in the treatment of the more extensive lesions, and endarterectomy in the short, well-localized lesions.

SUMMARY

1. Although extracranial atherosclerotic occlusive lesions may be demonstrated in 40 per cent of patients with cerebral arterial insufficiency, the functional significance of these lesions may be doubtful except in the more advanced cases. Although it may prove desirable to treat all of these lesions for prophylactic reasons, the best functional results will undoubtedly be obtained in the treatment of patients with functionally significant lesions. Local blood pressure changes in the region of disease, before, during, and after operation, carotid sinus activity, and cerebrospinal fluid pressure changes were studied in a group of patients to obtain observations which may be helpful both in the estimation of the functional significance of a lesion and in the conduct of reconstructive operation.

2. Occlusive lesions producing less than 47 per cent reduction in lumen diameter did not produce a blood pressure gradient. There was no other objective evidence of reduced blood flow in this group of patients. Since the vague, nonspecific symptoms were not relieved in these patients in whom the disease was removed, the lesions in these cases were considered functionally insignificant. Lesions producing more than 50 per cent reduction in lumen

diameter were associated with blood pressure gradients. This observation correlated with the clinical manifestation of the disease and certain physical evidence of alterations in blood flow. For this reason, these lesions were considered functionally significant.

3. Mean intra-arterial blood pressure measured distal to the region of temporary occlusion required for operation in patients with occlusion of the internal carotid and vertebral arteries varied from 12 to 90 mm. Hg, depending upon the presence of hypertension and contralateral occlusion. The lowest pressures were measured in patients with complete contralateral occlusion. Cerebral deficits occurred in these patients after brief periods of occlusion, indicating the need for support of circulation during operation. Although a pressure distal to operation was significantly raised by the administration of Neo-Synephrine solution, cerebral dysfunction was not prevented. Temporary internal shunts, however, provided a distal pressure equal to that obtained proximal to the occlusion and normal cerebral function was maintained by their application.

4. Bradycardia and hypotension frequently occurring during operation were not preventable by atropine sulfate. These disturbances occurred more frequently when general rather than local anesthesia was employed. Neo-Synephrine solution given intravenously invariably reversed the hypotension associated with carotid sinus activity and, for this reason, it is considered the therapeutic agent of choice.

5. Cerebral edema occurring in 3 patients was related to venous obstruction in the neck and mediastinum resulting from bilateral aortosubclavian bypass graft.

REFERENCES

1. Bakay, L., and Sweet, W. H.: Cervical and Intracranial Intra-arterial Pressures With and Without Vascular Occlusion, Surg. Gynec. & Obst. **95**: 67-75, 1952.
2. De Bakey, M. D., Crawford, E. S., Cooley, D. A., and Morris, G. C.: Surgical Considerations of Occlusive Disease of Innominate, Carotid and Subclavian Arteries, Ann. Surg. **149**: 690-710, 1959.
3. Halley, M. M., Reemsta, K., and Creech, O., Jr.: Hemodynamics and Metabolism of Individual Organs During Extracorporeal Circulation, SURGERY **46**: 1128-1134, 1959.
4. Mann, F. C., Herrick, J. F., Essex, H. E., and Baldes, E. J.: The Effect on the Blood Flow of Decreasing the Lumen of a Blood Vessel, SURGERY **4**: 249-252, 1938.

(For discussion, see page 109.)

RENAL REVASCULARIZATION FOR HYPERTENSION

Clinical and Physiologic Studies in 32 Cases

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FOR many years elucidation of physiologic mechanisms associating kidney disease and hypertension has both intrigued and eluded investigators. With unusual prescience, Bright[3] was the first to recognize this association clinically over 100 years ago. Mechanisms of the relationship, however, have not yet been fully established. The first clue in this regard was noted by Carrel[6] and Janeway[19] who found an elevation in blood pressure following surgical reduction of renal artery circulation. In 1934, Goldblatt, Lynch, Hanjal, and Summerville[16] published their classic experiments which marked the beginning of a period characterized by increasing studies concerning the pathogenesis of renovascular hypertension. Subsequent work, however, has shown that the Goldblatt type of hypertension in the dog is not necessarily permanent nor on an ischemic basis as originally described.[8-10, 15, 25, 29] The term "renal ischemic hypertension" is still used but without objective substantiating evidence. Current concepts hold that hypertension of experimental renal origin and human renal hypertension are probably initiated by an alteration in pulse pressure wave in the renal arteries followed by release of renin.[2, 5, 7, 13, 17, 20] Renin acts on a protein substrate from the liver to produce a decapeptide which in turn is converted to the highly pressor octapeptide angiotensin.[14, 24, 27] Needless to say, even in renal hypertension arterial pressure is the composite result of many both variable and interacting physiologic processes.

Despite a historically long and intense period of laboratory research regarding renal hypertension, clinical investigations have been relatively few, largely because of the unavailability of surgical means for definitive treatment other than nephrectomy.[22, 28] Recently techniques have been applied to correct the primary cause of hypertension resulting from renal artery stenosis by restoration of normal renal circulation with renal conservation rather than nephrectomy.[11, 12, 21, 22] The purpose of this report is to analyze certain physiologic measurements in 32 patients in whom renal revascularization was performed for unilateral or bilateral renal artery occlusive disease associated with hypertension, and to correlate these findings with previous concepts derived from experimental renal hypertension.

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MATERIAL AND METHODS OF STUDY

Thirty-two hypertensive patients with renal artery occlusive lesions, on whom revascularization procedures have been performed, constitute the basis of this study. Aorta to renal artery bypass graft was the most frequently employed technique in reconstituting normal hemodynamics (Fig. 1). Direct reconstruction of the renal artery with a gore or patch graft seemed to present ideal treatment in other selected cases. Knitted crimped Dacron was employed as the graft material in all cases. Etiological basis for renal artery stenosis appeared to be arteriosclerosis in every instance, with a patient age range from 35 to 70 years. Diagnosis was established by translumbar aortography with the use of 25 c.c. of 70 per cent Urokon as the contrast agent.[21, 23] Essential clinical statistics in this series are presented in Table I.

Various physiologic observations were made in these patients for 2 prime objectives. First it seemed desirable to correlate findings in human renovascular hypertension with previous observations in experimental hypertension. Second, an attempt has been made to evaluate proposed screening studies which might point to the candidature of certain hypertensive patients for aortography and definitive diagnosis.[4, 18, 31, 32] Physiologic observations of various patients include split and, in other cases, total renal function, renographic tracings, comparative excretory pyelography, and pulse wave recordings. Bilateral renal biopsies were obtained in some instances. Estimation of renal blood flow was made from renal plasma flow obtained by para-aminohippurate clearance. Inulin clearance was employed for measurement of glomerular filtration rate. Flame photometric techniques were used for determination of sodium concentration. Intravenous radioactive Diodrast with bilateral flank scanning constituted the essential methods of obtaining renograms. Pulse wave recordings were made with a pressure transducer at the time of operation by simultaneous needle puncture of the distal renal artery and the abdominal aorta.

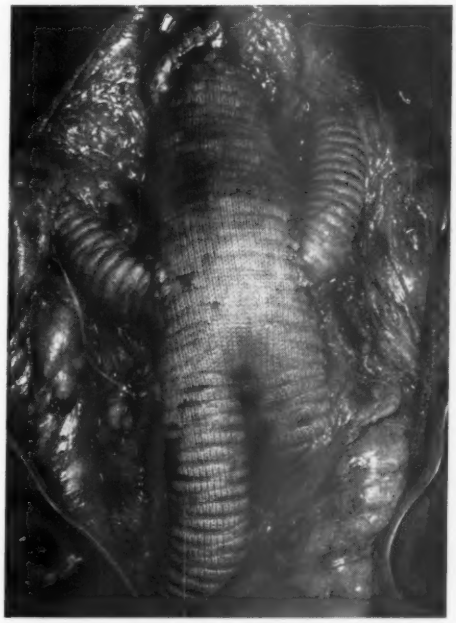
OBSERVATIONS AND RESULTS

Results of this surgical approach to renal artery stenosis with renal conservation through revascularization appear most gratifying. Eighty-one per cent of these patients have become normotensive since operation and have remained so for periods of 3 months to 2½ years. Amelioration of hypertension has occurred in all the remaining patients although some blood pressure elevation persists. Interestingly enough the 2 patients with the most modest improvement in blood pressure developed striking sensitivity to antihypertensive agents following operation. The only death in this series resulted from a massive coronary occlusion 5 days after operation (Patient 18, Table I).

Renal blood flow studies seem to corroborate experimental evidence pointing away from renal ischemia as a trigger mechanism in the production of renovascular hypertension. With but 1 exception, all patients had measured blood flow to the involved kidney well exceeding 200 ml. per minute (Fig. 2). In 6 instances, when differential blood flow was measured both before and after operation, a pronounced increase in blood flow after revascularization was seen in only 2 patients. Even in patients with extreme bilateral renal artery stenosis total renal blood flow appears only minimally depressed. In

Patient 3 (Fig. 3), for instance, glomerular filtration rate was 28 ml. per minute before operation and 98 after; renal blood flow was 650 ml. per minute before and 811 after; and blood pressure was 210/115 mm. Hg before and

A.



B.

C.

Fig. 1.—*A*, Aortogram performed before operation in 47-year-old hypertensive woman showing bilateral renal artery stenosis (Patient 14, Table I). Total renal blood flow was 954 ml. per minute. *B*, Photograph taken at operation showing origin of renal artery bypass grafts from aorta to external iliac bypass employed for concomitant correction of aortoiliac occlusive disease. *C*, Aortogram performed after operation showing bilateral renal artery bypass grafts. Renal blood flow did not change significantly and was 1,030 ml. per minute after operation.

TABLE I

NO.	PATIENT	AGE	SEX	DURATION OF HYPERTENSION	BLOOD PRESSURE		SIDE OF LESION		TECHNIQUE OF REVASCULARIZATION	ASSOCIATED VASCULAR PROCEDURE
					BEFORE	AFTER	RIGHT	LEFT		
1.	C. S.	51	F	2 mo.	230/120	120/80	X		Right aortorenal graft bypass	Endarterectomy of distal aorta and common iliac arteries
2.	A. N.	43	M	3 mo.	220/100	136/86	X		Right aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
3.	M. G.	45	F	10 yr.	220/120	130/70	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
4.	G. J.	58	M	1 yr.	200/110	140/80		X	Left aortorenal graft bypass	Aorto-bilateral femoral graft bypass
5.	L. W.	51	F	1 yr.	210/115	150/85	X	X	Right aortorenal graft bypass; left renal endarterectomy with patch graft	None
6.	W. D.	59	M	Indeterminate	180/120	170/90	X	X	Bilateral aortorenal graft bypass	Excision of abdominal aortic aneurysm and graft replacement
7.	S. G.	53	M	1 yr.	190/130	180/108	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral femoral graft bypass
8.	L. R.	44	F	4 yr.	200/140	140/70	X	X	Bilateral aortorenal graft bypass	None
9.	K. H.	44	M	7 mo.	215/130	130/80		X	Left renal artery patch graft reconstruction	None
10.	C. B.	50	M	2 mo.	240/130	180/90	X		Right aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
11.	H. G.	64	M	1 yr.	160/100	135/70		X	Left aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
12.	I. B.	70	M	5 yr.	170/90	130/70	X		Right aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
13.	J. C.	45	M	3 yr.	200/110	110/70	X	X	Right aortorenal graft bypass; left nephrectomy	None
14.	A. A.	47	F	8 yr.	170/90	114/74	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
15.	L. N.	55	M	2 yr.	160/90	120/70		X	Left aortorenal graft bypass	Aorto-bilateral femoral graft bypass
16.	L. T.	62	F	5 yr.	224/114	150/80	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral femoral popliteal graft bypass

TABLE I—CONT'D

NO.	PATIENT	AGE	SEX	DURATION OF HYPERTENSION	BLOOD PRESSURE		SIDE OF LESION		TECHNIQUE OF REVASCULARIZATION	ASSOCIATED VASCULAR PROCEDURE
					BEFORE	AFTER	RIGHT	LEFT		
17.	C. T.	55	F	6 yr.	180/100	130/80	X		Right renal artery patch graft reconstruction	Aortic resection with right common iliac, left external iliac graft bypass
18.	P. S.	67	M	2 yr.	180/100	190/80		X	Left aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
19.	N. B.	47	F	23 yr.	210/120	140/72	X	X	Bilateral aortorenal graft bypass	None
20.	H. W.	55	M	7 yr.	210/120	170/86		X	Left aortorenal graft bypass	Aorto-bilateral femoral graft bypass
21.	J. P.	35	M	9 mo.	200/115	120/70	X		Right renal endarterectomy with patch graft reconstruction	None
22.	C. J.	35	F	3 yr.	170/130	110/70	X		Right aortorenal graft bypass	None
23.	W. T.	57	M	7 mo.	192/106	140/70		X	Left aortorenal graft bypass	Aorto-bilateral femoral popliteal graft bypass
24.	L. C.	39	M	Indeterminate	190/110	120/80		X	Left renal artery patch graft reconstruction	None
25.	A. A.	64	F	10 yr.	210/120	160/80		X	Left aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
26.	J. B.	35	M	10 yr.	250/125	120/80	X	X	Resection and graft replacement right renal artery; left nephrectomy	None
27.	E. C.	50	M	Indeterminate	200/100	120/80	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral femoral graft bypass
28.	M. N.	37	M	4 yr.	220/130	140/80		X	Left aortorenal graft bypass	None
29.	B. C.	56	M	2 yr.	250/130	110/80	X	X	Bilateral aortorenal graft bypass	Aorto-bilateral external iliac graft bypass
30.	I. M.	56	F	20 yr.	250/120	120/80	X		Right renal endarterectomy through the aorta	Excision abdominal aortic aneurysm and graft replacement
31.	D. M.	49	M	2 yr.	230/160	135/85	X	X	Right aortorenal graft bypass; left nephrectomy	Aorto-bilateral external iliac graft bypass
32.	L. J.	55	M	7 yr.	250/125	140/85	X	X	Bilateral renal endarterectomy and patch graft reconstruction	None

130/70 after. Despite a severe degree of bilateral renal artery stenosis, renal blood flow was only moderately reduced before operation.

In unilateral stenosis where hypertension has not been of long duration the uninvolved kidney usually functions well before operation while afterward there is a fall in function below that of the revascularized kidney (Fig. 2). Reduced filtration pressure after operation with nephrosclerotic changes together seem pertinent to this phenomenon. Serial studies have, in general,

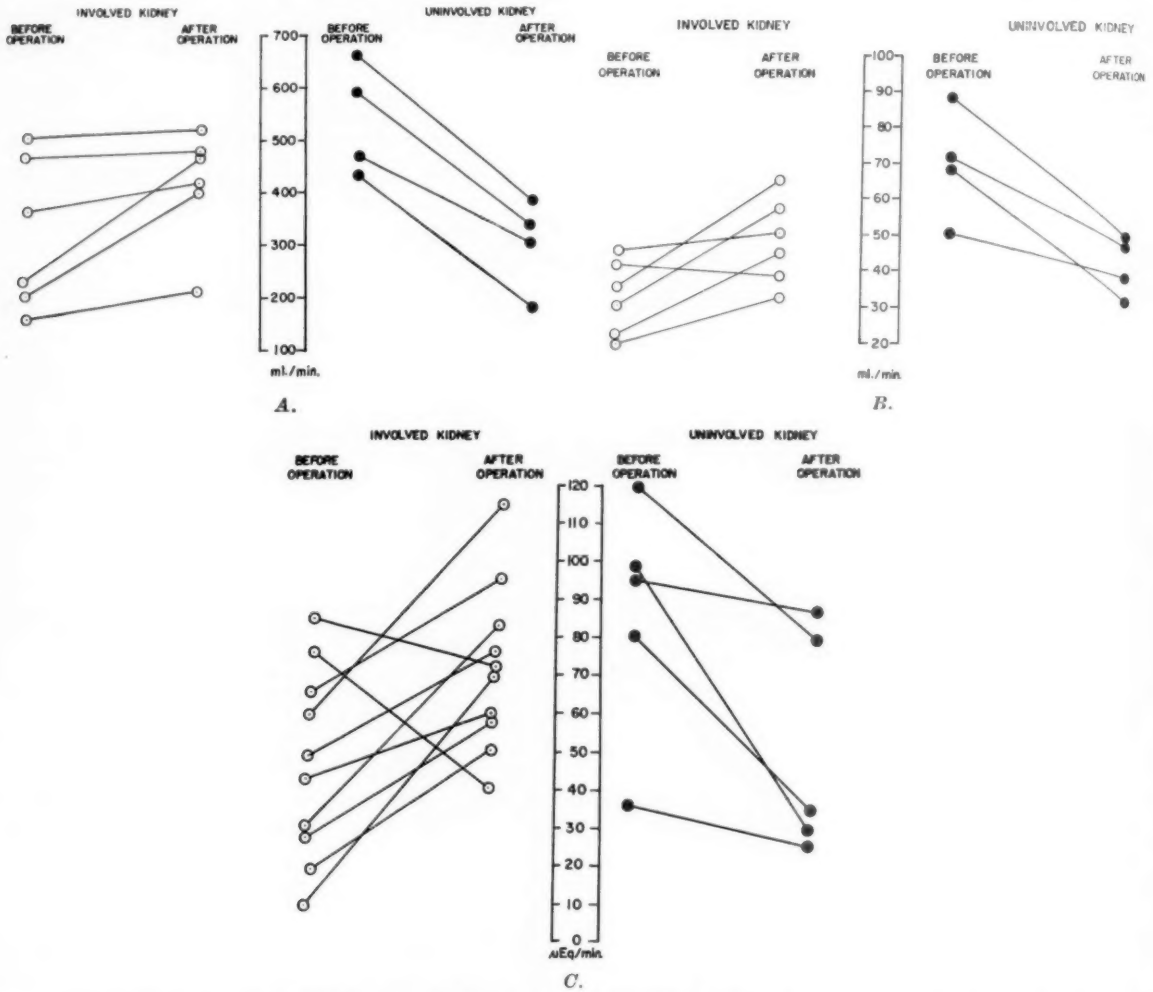


Fig. 2.—*A*, Differential renal blood flow studies before and after revascularization. Only 2 revascularized kidneys showed significant increase in blood flow while all uninvolved kidneys demonstrated reduced blood flow following operation. *B*, Differential glomerular filtration studies before and after revascularization. Glomerular filtration usually increased in response to revascularization while in unilateral cases this function always decreased on the uninvolved side. *C*, Sodium excretion was variably reduced on the side of stenosis and generally responded to revascularization with significantly increased excretion. Sodium excretion was reduced from the uninvolved kidney following unilateral revascularization. Urine sodium concentration usually rose on the revascularized side and remained unchanged in the uninvolved kidney.

shown continued improvement on the side of revascularization and late secondary improvement on the uninvolved or minimally involved kidney (Figs. 4 and 5). This late secondary improvement suggests some reversibility of the nephrosclerotic process concurrent with cure of hypertension.

Renographic tracings usually have shown flattening of the counting curve commensurate with the degree of renal artery stenosis. With severe stenosis initial uptake is slower indicating reduction in vascularity, and the absorption-secretion phase is prolonged suggesting suppression of tubular efficiency (Fig. 6). Following operation the revascularized kidney has shown return to predicted normal curve and in unilateral cases the uninvolved kidney loses its apparent exaggerated function.

A majority of excretory pyelograms in these cases showed no discernible abnormalities. Abnormal findings, when present, varied from slight reduction in renal size on the side of the lesion associated with normal excretion, through delayed excretion, to apparent nonfunction. In 3 patients, who had no excretion on intravenous pyelography, renal function was restored. These instances emphasize the importance of complete evaluation with arteriography before nephrectomy is carried out (Figs. 7 and 8).

Pulse wave tracings were obtained before and after revascularization in only a few cases. These have shown variable alterations in the contour and

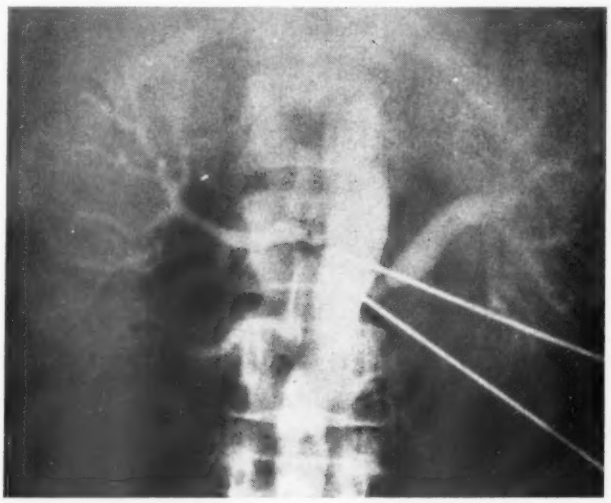


Fig. 3.—Renal arteriogram showing extreme degree of bilateral renal artery stenosis. An accessory artery supplies lower pole of right kidney (Patient 3, Table I).

amplitude of the pulse pressure wave, varying from slight dampening of the systolic notch to near flattening of the wave and significant reduction in mean blood pressure (Fig. 9). Following revascularization the renal artery pulse waves became indistinguishable from aortic pulse waves.

DISCUSSION

Although blood pressure control is a complex mechanism depending on many variable factors, the gratifying response of hypertension to revascularization in these 32 cases firmly establishes this method of treatment for patients with stenotic lesions of the renal artery. Review of the 6 patients whose response was incomplete has produced several speculative reasons for failure of blood pressure to return to normal. These include, first, partial irreversibility of arteriolar changes resulting from pre-existing hypertension

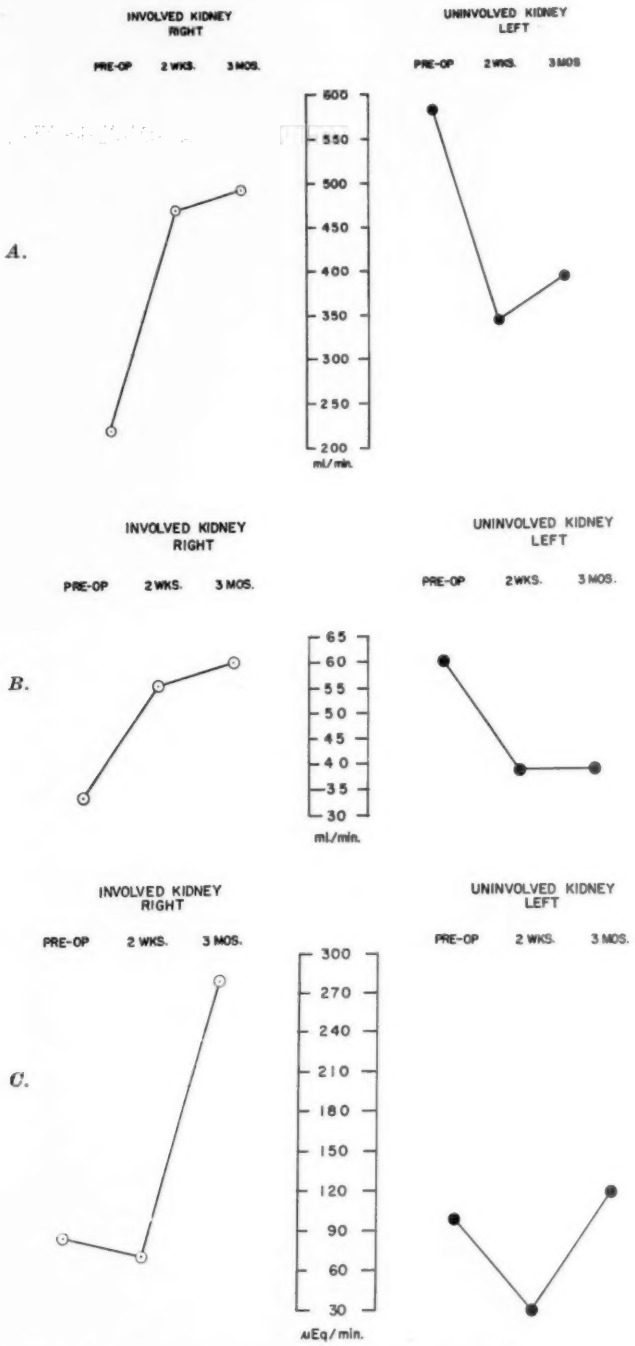


Fig. 4.—*A*, Effect of unilateral revascularization on differential renal blood flow (Patient 22, Table I). Sudden reduction in arterial pressure appears pertinent to initial reduction in renal blood flow in the uninvolved kidney. *B*, Effect of unilateral revascularization on glomerular filtration rate. Persistence of reduced function in the uninvolved kidney may be related to nephrosclerosis produced by previous hypertension. *C*, Comparative sodium excretions in this case with right renal artery stenosis showed little difference before operation. Sodium concentration did show characteristic depression on the right and concentration increased progressively after operation.

in the contralateral kidney without renal artery stenosis.[1, 26, 28, 30, 33] Two other possibilities in this group are an overlooked minimal stenosis on the side opposite reconstruction and unresolved systolic hypertension from generalized arteriosclerosis. Contralateral nephrectomy was performed in 3 instances but in each it was carried out because of complete occlusion of the renal artery with distal thrombosis or because of extensive arteriosclerotic involvement of the renal artery making reconstruction impossible from a technical standpoint. As yet it has not appeared justified to perform contralateral nephrectomy on the unstenosed side because of advanced arteriolar

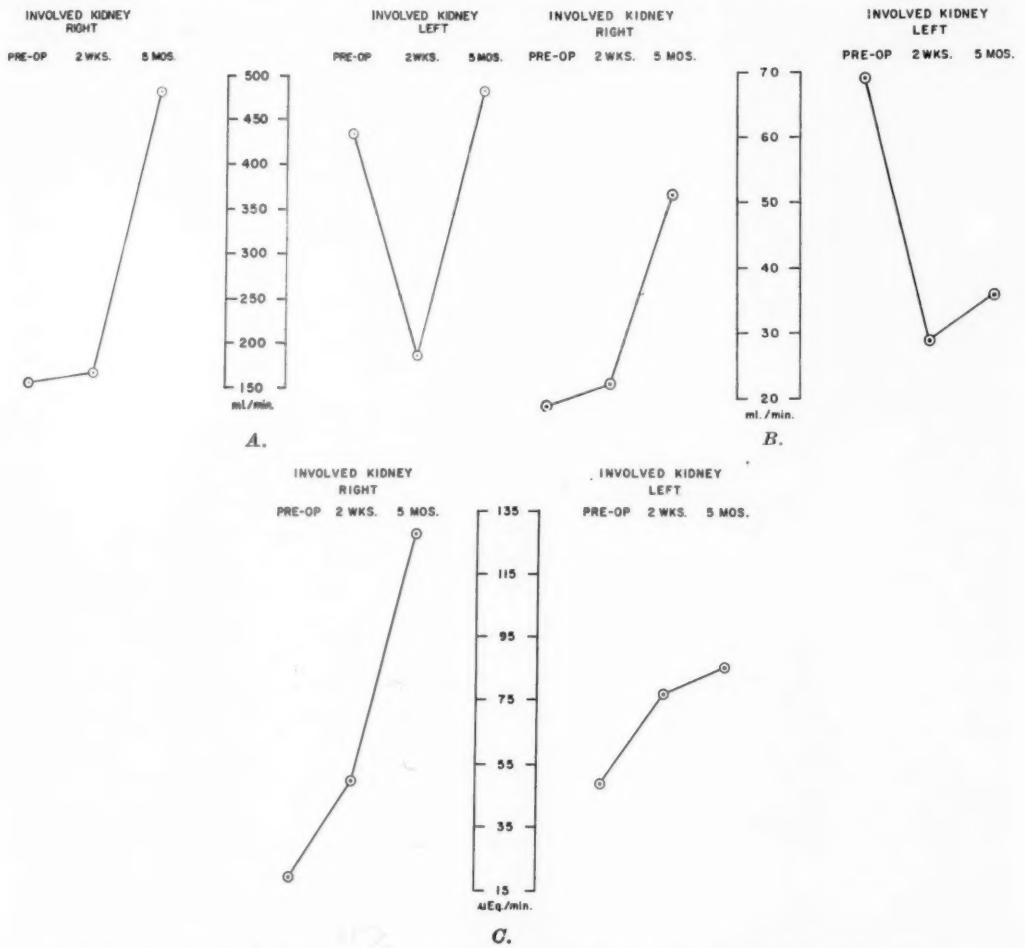
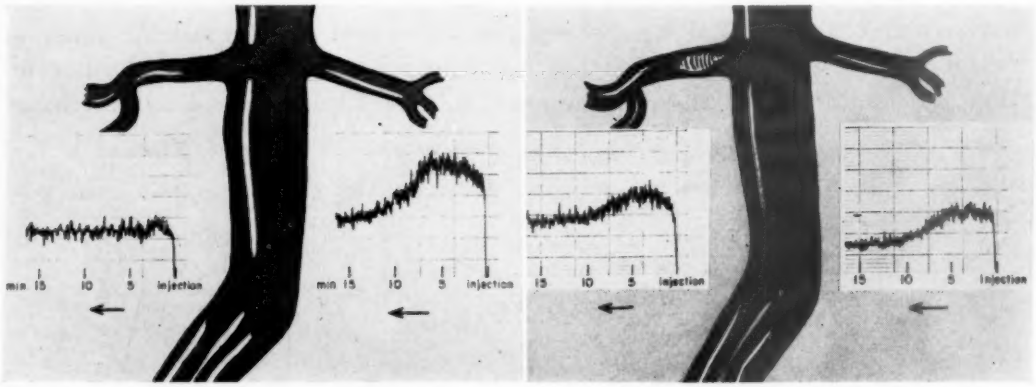


Fig. 5.—*A*, Effect of bilateral renal revascularization on renal blood flow where stenosis is marked on right and minimal on left (Patient 8, Table I). *B*, Effect of bilateral renal revascularization on glomerular filtration. Left side with minimal stenosis has response similar to uninvolved kidney following contralateral revascularization. *C*, Sodium excretion rises progressively after operation on both sides. Sodium concentration also had a similar bilateral increase following revascularization.

changes. Despite significant nephrosclerotic changes in the contralateral kidney, most of the patients in this series with unilateral renal artery disease became normotensive following revascularization. Delayed response of the blood pressure in some of these cases, occasionally requiring several months to return to normotensive levels, might well reflect partially reversible arteriolar changes on the side not operated upon (Fig. 10). With only 2 exceptions bilateral

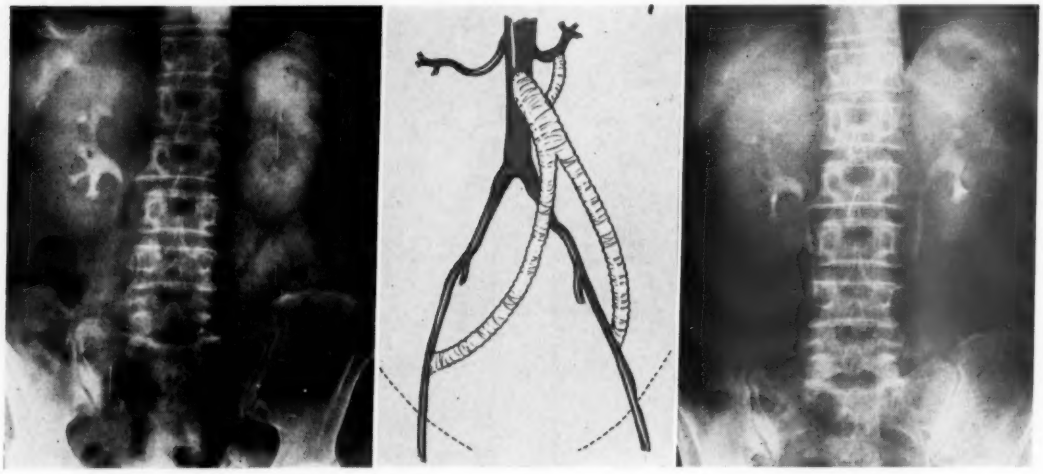
revascularization or unilateral revascularization and contralateral nephrectomy have resulted in an immediate and sustained normal blood pressure. Bilateral renovascular lesions were treated in 44 per cent of the cases in this series.

From a functional standpoint these studies also show the revascularized kidney in unilateral cases to be the superior kidney, apparently having been



A. *B.*

Fig. 6.—*A*, Comparative renogram tracings following intravenous injection of radioactive Diodrast in patient with right renal artery stenosis. *B*, Renogram tracings after right renal artery endarterectomy and patch graft reconstruction. Revascularized right kidney presents more normal curve while contralateral kidney has somewhat flatter curve after operation.



A. *B.* *C.*

Fig. 7.—*A*, Excretory pyelogram in patient with left renal artery stenosis showing delayed excretion on side of lesion (Patient 25, Table I). *B*, Drawing showing technique of aorta to external iliac bypass bilaterally with left renal artery bypass. *C*, Excretory pyelogram following revascularization showing normal excretion on left side. Both roentgenograms were made 10 minutes after intravenous injection of contrast medium.

protected by its own narrowed artery from the damaging effects of hypertension (Fig. 10). Late differential function studies also suggest some reversibility in the kidney damaged by hypertension (Figs. 4 and 5). Thus it would appear wise to defer decision on contralateral nephrectomy for at least several months following unilateral revascularization.

Renal arteriography is the only technique for making a definitive diagnosis of renal artery disease. Differential renal function studies cannot distinguish bilateral from unilateral disease nor primary renal artery branch lesions from

pyelonephritis.[13] From a clinical standpoint these studies seem to have their greatest value in an adjunctive or confirmatory capacity. Although renal arteriography can be performed with a high degree of quality and with accurate

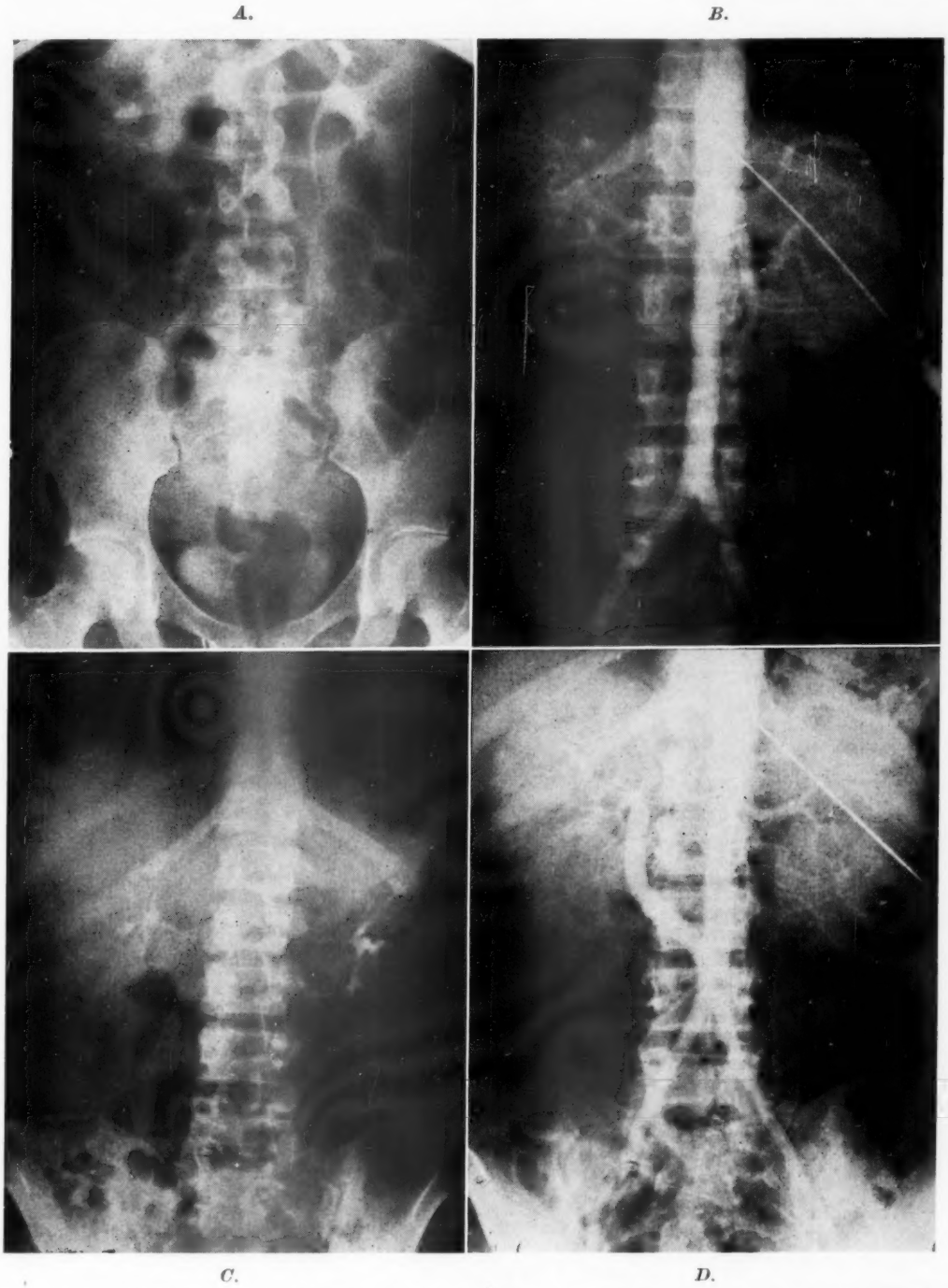


Fig. 8.—*A*, Excretory pyelogram in 35-year-old woman with right renal artery stenosis showing no visualization of right kidney (Patient 22, Table I). *B*, Aortogram showing long area of stenosis involving major segment of right renal artery. *C*, Excretory pyelogram after operation showing normal function in right kidney. *D*, Aortogram following operation showing right renal artery bypass. Large primary branch was used for graft attachment. Note increased size of right kidney following revascularization.

delineation of the renal arteries, it is still sometimes difficult to appraise orificial stenosis at the artery's origin, especially when the renal artery originates somewhat posteriorly from the aorta. Under such circumstances various differential function studies may be helpful (Fig. 11). At the present time no simple screening study for renovascular hypertension exists. Blood pressure response to intravenous tetraethylammonium chloride as a means of differentiating neuro-

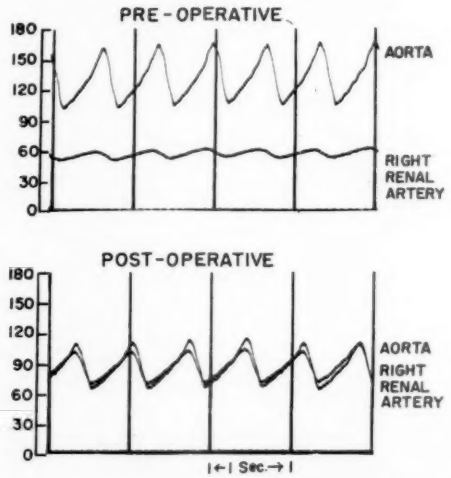
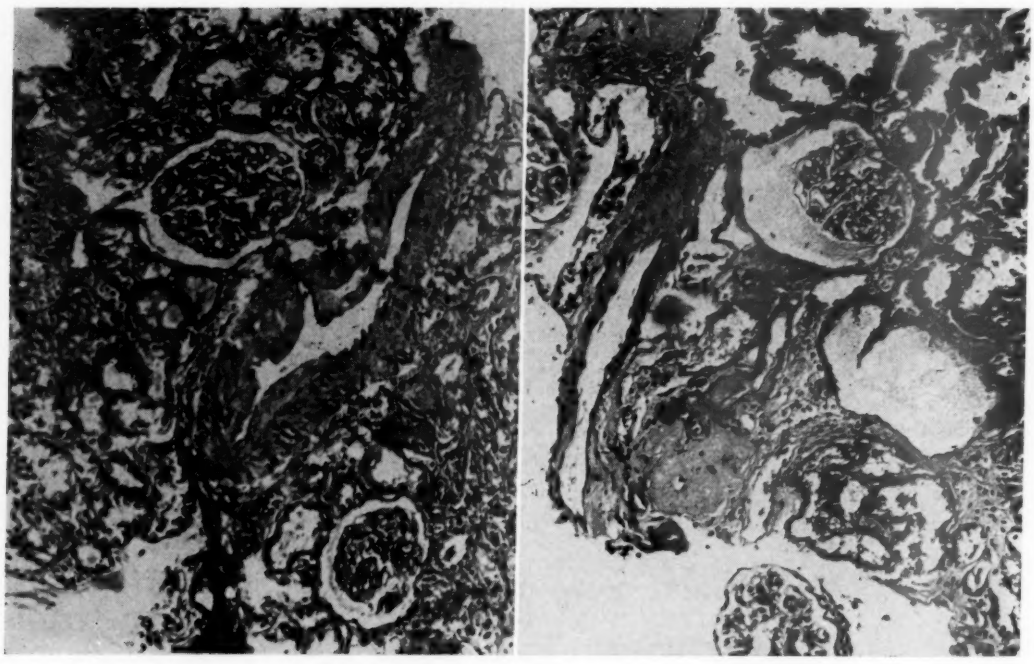


Fig. 9.—Pulse pressure tracings in patient with right renal artery stenosis before and after endarterectomy and patch graft reconstruction (Patient 21, Table I).



A. *B.*

Fig. 10.—*A*, Photomicrograph obtained from needle biopsy right kidney in patient with unilataeral right renal artery stenosis. Arterioles and glomeruli are essentially normal (Patient 22, Table I). *B*, Photomicrograph obtained from needle biopsy of left kidney with normal renal artery. Note advanced arteriolar and glomerular changes produced by hypertension.

genic from renal humoral hypertension has been disappointing.[4] Renal arteriography appears justified as an essential diagnostic procedure whenever severe hypertension exists without apparent cause, such as pyelonephritis. Aortography of the type used for renal artery visualization now appears safe in light of our most recent experience without death or serious complication in 1,200 consecutive patients.

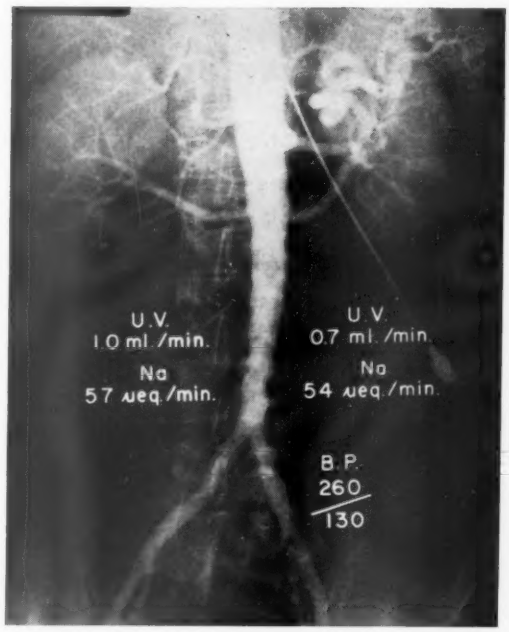


Fig. 11.—Aortogram and split function studies in hypertensive patient with renogram suggesting possible renal vascular lesion on right. Aortogram showed normal left renal artery but suggested possible orificial stenosis on right. Equal split function helped decision for nonoperative treatment in this case.

SUMMARY

This study was based on a surgical experience with 32 hypertensive patients on whom renal revascularization procedures were performed for renal artery stenosis.

Renal artery bypass with a graft was the most frequently employed technique in revascularization. In selected cases, direct reconstruction of the narrowed segment of the renal artery was performed with a patch graft.

Eighty-one per cent of these patients developed normal blood pressure after operation. Nineteen per cent were improved but demonstrated some residual hypertension. Renal blood flow studies seemed to corroborate experimental evidence pointing away from renal ischemia as a trigger mechanism in the production of renovascular hypertension.

In unilateral renal artery stenosis, the kidney distal to the occlusive process becomes the better kidney following revascularization from both a functional and pathologic standpoint. Severe hypertension without obvious cause, such as pyelonephritis, warrants renal arteriography, which is the only method for establishing the diagnosis of renovascular hypertension.

ADDENDUM

Since submission of this report an additional 28 hypertensive patients have had unilateral or bilateral renal revascularization procedures. The ages in 3 of these patients were 24, 27, and 29 years. Normal blood pressure has been restored in 82 per cent of these 60 patients.

REFERENCES

1. Bauer, H., and Forbes, L.: Unilateral Renal Artery Obstruction Associated With Malignant Nephrosclerosis Confined to the Opposite Kidney, Am. Heart J. **44**: 634, 1952.
2. Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., and Munoz, J. M.: La substance hypertensive extraite du sang des reins ischémies, Compt. rend. Soc. biol. **133**: 731, 1940; Substance Causing Renal Hypertension, J. Physiol. **98**: 282, 1940.
3. Bright, Richard: Guy's Hosp. Rep. **1**: 380, 1846.
4. Brust, A. A., and Ferris, E. B.: The Diagnostic Approach to Hypertension Due to Unilateral Kidney Disease, Ann. Int. Med. **47**: 1049, 1957.
5. Bumpus, F. M., Schwartz, H., and Page, I. H.: Synthesis and Pharmacology of the Octapeptide Angiotonin, Science **125**: 886, 1957.
6. Carrel, Alexis: Note on the Production of Kidney Insufficiency by Reduction of the Arterial Circulation of the Kidney, Proc. Soc. Exper. Biol. & Med. **6**: 107, 1909.
7. Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H.: Changes of Arterial Blood Pressure and Renal Hemodynamics by Injection of Angiotonin in Human Beings, Proc. Soc. Exper. Biol. & Med. **46**: 244, 1941.
8. Corcoran, A. C., and Page, I. H.: Renal Blood Flow in Experimental Hypertension Due to Constriction of the Renal Artery, Am. J. Physiol. **133**: 249, 1941.
9. Corcoran, A. C., and Page, I. H.: Renal Blood Flow in Experimental Renal Hypertension, Am. J. Physiol. **135**: 361, 1942.
10. Daniel, P. M., Prichard, M. M., and Ward-McQuaid, J. N.: An Angiographic Study of the Renal Circulation in Experimental Hypertension in the Dog, Brit. J. Surg. **42**: 212, 1954.
11. DeCamp, P. T.: Renal Artery Hypertension; A Correctable Clinical Condition, Dis. Chest **34**: 640, 1958.
12. DeCamp, P. T., and Birchell, R.: Recognition and Treatment of Renal Arterial Stenosis Associated With Hypertension, SURGERY **43**: 134, 1958.
13. Dustan, H. P., Page, I. H., and Poutasse, E. F.: Renal Hypertension, New England J. Med. **261**: 647, 1959.
14. Elliott, D. F., and Peart, W. S.: Amino Acid Sequence in a Hypertensin, Nature **177**: 527, 1956.
15. Farrell, J. I., and Young, R. H.: Hypertension Caused by Unilateral Renal Compression, J. A. M. A. **118**: 711, 1942.
16. Goldblatt, H., Lynch, J., Hanjal, R. F., and Summerville, W. W.: Studies on Experimental Hypertension: Production of Persistent Elevation of Systolic Blood Pressure by Means of Renal Ischemia, J. Exper. Med. **59**: 347, 1934.
17. Hawthorne, E. W., Perry, S. L. C., and Pogue, W. G.: Development of Experimental Renal Hypertension in the Dog Following Reduction of Renal Artery Pulse Pressure Without Reducing Mean Pressure, Am. J. Physiol. **174**: 393, 1953.
18. Howard, J. E., Berthrong, M., Gould, D. M., and Yendt, E. R.: Hypertension Resulting From Unilateral Renal Vascular Disease and Its Relief by Nephrectomy, Bull. Johns Hopkins Hosp. **94**: 51, 1954.
19. Janeway, T. C.: Note on the Blood Pressure Changes Following Reduction of the Renal Artery Circulation, Proc. Soc. Exper. Biol. & Med. **6**: 109, 1909.
20. Kohlstaedt, K. G., and Page, I. H.: Liberation of Renin by Perfusion of Kidneys Following Reduction of Pulse Pressure, J. Exper. Med. **72**: 206, 1940.
21. Morris, G. C., Jr., De Bakey, M. E., Cooley, D. A., and Crawford, E. S.: Surgical Treatment of Renal Hypertension, Ann. Surg. In press.
22. Poutasse, E. F.: Surgical Treatment of Renal Hypertension: Results in Patients With Occlusive Disease of Renal Arteries, J. Urol. **82**: 403, 1959.
23. Poutasse, E. F., Engel, W. J., and Root, J. C.: Translumbar Aortography, Cleveland Clin. Quart. **19**: 105, 1952.
24. Rittel, W., Iselin, B., Kappeler, H., Riniker, B., and Schwyzer, R.: Synthese eines. hochwirksamen Hypertensin II—amids (L-Asparaginyl-L-arginyl-L-valyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanin), Helvet. chim. acta **40**: 614, 1957.
25. Schroeder, H. A., and Steele, J. M.: The Behavior of Renal Blood Flow After Partial Constriction of the Renal Artery, J. Exper. Med. **72**: 707, 1940.
26. Schwartz, N. H., and Gross, S.: Unilateral Malignant Nephrosclerosis, J. Urol. **62**: 426, 1949.

27. Skeggs, L. T., Jr., Kahn, J. R., and Shumway, N. P.: Preparation and Function of Hypertensin-Converting Enzyme, J. Exper. Med. 103: 295, 1956.
28. Smith, H. W.: Hypertension and Urologic Disease, Am. J. Med. 4: 724, 1948.
29. Warthin, T. A., and Thomas, C. B.: Studies in Experimental Hypertension. Phenol Red Excretion and Renal Blood Flow in Hypertension of Renal Origin, Bull. Johns Hopkins Hosp. 72: 203, 1943.
30. Wilson, C., and Byrom, F. B.: Viscous Circle in Chronic Bright's Disease: Experimental Evidence From Hypertensive Rat, Quart. J. Med. 10: 65, 1941.
31. Winter, C. C.: A Clinical Study of a New Renal Function Test: The Radioactive Diodrast Renogram, J. Urol. 76: 182, 1956.
32. Winter, C. C., Maxwell, M. H., Rockney, R. E., and Kleeman, C. R.: Results of Radioactive Renogram and Comparison With Other Kidney Tests, J. Urol. 82: 674, 1959.
33. Yuile, C. L.: Obstructive Lesion of Main Renal Artery in Relation to Hypertension, Am. J. M. Sc. 207: 394, 1944.

Discussion

(Papers by Crawford, De Bakey, and Morris; Morris, Cooley, Crawford, Berry, and De Bakey)

DR. PAUL T. DE CAMP (New Orleans, La.).—Four years ago we presented here our initial results in one of the earliest cases of revascularization of the kidney. This occurred in a little girl 10 years old with only 1 kidney. She has gone an additional 4 years with a normal blood pressure—entirely well. It is difficult to make a definition of a disease; however, we feel that this condition should be defined, so we suggest renal arterial stenosis causing significant changes in renal or systemic physiology. The renal physiology is the abnormal reabsorption of water and sodium. The systemic physiologic changes are two: (1) a very significant rise in hypertension, almost always in the diastolic phase, and (2) extreme nervousness of the patient. The inner tension and restlessness we have seen regularly. I think you will realize before I am through that we are apparently seeing a little different disease in New Orleans than they are in Houston. But the patients with this disease whom we accept have all had a marked inner tension and restlessness and the remarkable thing is that it is relieved immediately after the operation even though the blood pressure may come down more slowly. It suggests strongly that this is a phase of the humoral reaction which is promptly relieved although the fixed pattern of hypertension may be relieved more slowly. Because both sodium and water are reabsorbed one cannot, by measuring the concentration of the sodium, i.e., relating it to concentration of water, arrive at any valuable information from merely a sample of urine. However, if the concentration of sodium is compared with a substance such as creatinine, which is not reabsorbed, then merely a sample of urine will be sufficient to give the tip-off, and if one obtains the plasma level one will recognize the change even in bilateral disease. In the cases of this condition which we have accepted, we have always demonstrated a very significant difference in the mean pressure proximal and distal to the point of obstruction. As you will see, here in the aorta in the right renal artery the mean pressure is 130 odd, and distal to the stenosis in the left renal artery the pressure is 75. In fact, in none of our cases has there been a mean pressure of less than 50 mg. of differential. I do not quite understand how significant a little bit of difference of pressure curve could be if the patient is hypertensive beyond the area of the stenosis.

DR. RALPH A. DETERLING, JR. (Boston, Mass.).—I thoroughly agree with Dr. Crawford's comments on the value of an external shunt in carotid surgery. To illustrate this, I wish to describe a patient with a large syphilitic aneurysm of the right common carotid artery. Clot from the sac had passed to the brain with a resulting hemiplegia. At the time of resection of the aneurysm, the patient had partially recovered with the use of the external shunt. We were able to remove the aneurysm and insert a synthetic graft without further worsening of his weakness. The shunt consisted of a short length of plastic intravenous tubing fitted to two 15 gauge needles.

DR. FRANK C. SPENCER (Baltimore, Md.).—My comments concern Dr. Crawford's paper. I much enjoyed his presentation and would like to comment on 2 points. Concerning the origin of the cerebral insufficiency, we have considered it to be due to the decreased cerebral blood flow and do not have any conclusive findings indicating otherwise. Two or 3 patients, however, have had on the atheromatous plaques small accumulations of platelets or fibrin. These are very friable, can be dislodged readily, and raise the question of whether symptoms might be due to recurrent emboli. Recurrent emboli could be the reason why people develop symptoms while at rest or in bed or in other conditions where one would not expect a spontaneous change in blood flow. This consideration has some importance because it may influence future decisions for operation and not limit it completely to patients who have a severe stenosis of the carotid artery.

The other point I would like to make is to strongly endorse the use of local anesthesia for the performance of carotid endarterectomy. We have routinely used this for the past year in about 20 patients, and have been unable to predict ahead of time whether a shunt would be needed or not. Eighteen of the 20 patients tolerated 10 minutes of occlusion of the carotid artery without difficulty. In 2 of the 20, however, less than 1 minute of carotid occlusion caused a frightening hemiplegia and coma, promptly relieved when the clamps were released. A temporary shunt was used in these 2 patients while an endarterectomy was performed. One of these patients, on subsequent study, did not have any demonstrable disease in the contralateral carotid artery, so we had no explanation for the absence of collateral circulation.

DR. LEON GOLDMAN (San Francisco, Calif.).—I would like to take this opportunity to report on 3 patients who have been seen during the past year by Dr. Edwin J. Wylie on our Vascular Service. These patients were among the 18 who were operated on for hypertension due to stenosis of the renal artery. Only 6 similar cases have been cited in the literature, and the report of these 3 will probably be of some interest to this Society.

All 3 patients were women in their fourth decade who developed hypertension during pregnancy. One was a patient who had had stenosis of both left renal arteries, proximal aneurysmal dilatation, and positive evidence of renal artery stenosis as demonstrated by the John Eager Howard test. The narrow segment in the midportion of the renal artery can be seen on the slide, and is in contradistinction to the narrowing found in obliterative endarteritis, which usually occurs where the renal artery branches off from the abdominal aorta. The slides show cross sections of the renal arteries, which were resected, and an arterial anastomosis was performed in these 2 patients. The arteries showed marked thickening of the media, fibromuscular hyperplasia and cellular infiltration of the media, with some fragmentation of the elastic tissue and resultant narrowing of the lumen. The special stain shows both the fibrous and smooth muscle components. The slide of the second patient shows a sausagelike beading effect of the renal artery, with aneurysmal dilatation extending into the secondary radicals and even into the kidney itself. In this particular instance nephrectomy was necessary, and the patient's hypertension has not returned postoperatively. The last slide is of a section taken through these aneurysmal areas which shows thinning of the opposite wall and fibromuscular hyperplasia of the media.

DR. MORRIS (closing).—I am indeed grateful to Dr. DeCamp and Dr. Goldman for their discussion. Emotional lability or nervousness noted so prominently by Dr. DeCamp has not appeared to be a very significant clinical feature in our patients. With reference to etiology, our patient age range varied from 35 to 70 years and the basic lesion seemed to be arteriosclerosis in all cases. Three patients were 35 years of age and each of these showed lesions similar to Dr. Goldman's patient with so-called juvenile atherosclerosis. The stenotic process in these very young individuals is produced by thickening of the intima and media by fibromuscular proliferation.

STUDIES IN ISOLATED ORGAN PHYSIOLOGY: BROMSULPHALEIN CLEARANCE IN THE ISOLATED PERFUSED BOVINE LIVER

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THE secretion of bile is a complex process that is poorly understood in spite of a large volume of experimental work. As a model excretory product appearing in bile, Bromsulphalein (BSP) has been extensively studied in its transfer from blood to bile.

Bile BSP has been variously described as a secretion of the hepatic parenchymal cells,[2,3] or as a filtration-resorption product of the bile ducts.[1] In this latter scheme, the peribiliary arterial plexus is said to supply BSP and other important substances directly to the bile duct cells for excretion. This concept of intrahepatic excretory processes is based upon studies conducted in perfused canine livers, interpreted as showing preferential uptake of Bromsulphalein by the hepatic arterial circulation.

The lack of agreement concerning the mechanism of hepatic BSP excretion can be attributed largely to the deficiency of knowledge of the functional anatomy of the bile secretory apparatus and the difficulty in establishing a standardized biologic preparation in which variables relating to the secretion and composition of bile can be studied.

This laboratory has developed an isolated, artificially perfused liver preparation utilizing the bovine liver. With this reproducible biologic preparation, it is now possible to continue satisfactory perfusion for periods of up to 24 hours. Observations of BSP clearance have been made when the isolated liver was selectively perfused, either entirely through the hepatic artery or entirely through the portal vein, to determine if a preferential arterial route of excretion for this substance was present.

METHODS

The methods employed in these experiments (details of which will be published elsewhere[11]) include the use of an apparatus which circulates, through 2 Sigmamotor peristaltic pumps, oxygenated blood from a reservoir to the hepatic artery and portal vein of the liver and which allows venous blood from the liver to return freely by gravity flow to the oxygenator. A total volume of 2 to 2.5 L. of perfusate (blood diluted with modified Ringer's solution) with a hematocrit of 25 to 30 was pumped through the hepatic artery and portal vein at usual flows in each vessel of 100 ml. per minute and mean pressures

Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

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of 100 mm. Hg in the hepatic artery and 12 cm. H_2O in the portal vein. In some experiments, flow rates in each vessel were varied from 80 to 215 ml. per minute with corresponding maximum pressures of 240 mm. Hg for the hepatic artery and 30 cm. H_2O for the portal vein. The use of aseptic surgical techniques combined with the use of a 0.5 per cent formalin solution to cold-sterilize some parts of the perfusion apparatus, with steam sterilization of others, has allowed maintenance of apparent bacterial sterility up to 12 hours. All preparations were monitored for bacterial contamination. The perfusate temperature was maintained at 37.0° C. ± 0.5, and pH was adjusted between 7.2 and 7.5 by regulating the proportions of oxygen and carbon dioxide entering the bubble oxygenator.

Bromsulphalein, at a dose of 0.1 to 0.3 mg. per gram liver (wet weight), was injected directly into the inflow vessel or vessels just before entry into the liver, or into the venous blood from the isolated organ just before return to the oxygenator and reservoir. Sampling was carried out under sterile conditions with syringe and needle through gum rubber sections of the circulation vessels.

The removal of Bromsulphalein was also studied in "intact" anesthetized animals. In some of these experiments, the hepatic artery was isolated and occluded temporarily at the hilus. Clearances were determined when the liver was supplied with blood either through the portal vein alone or through both the portal vein and the hepatic artery. BSP was injected into a peripheral vein and samples were withdrawn from the vena cava.

The concentrations in milligrams per cent of Bromsulphalein in the plasma samples were measured by the method of Gaebler.[14] A semilog plot of these values against time was made and a straight line fitted to the plotted points. The slope of the line was then determined after the method of Ingelfinger and associates.[18]*

The plasma disappearance rates calculated from experiments in which Bromsulphalein was injected into the hepatic artery or portal vein are not strictly comparable to the plasma disappearance rates (PDR) of Ingelfinger and co-workers. This is so because the calculations were made only for the first 5 minutes following injection, during which time *both* mixing and clearance were occurring.

In the "intact" animals and also in those isolated liver experiments in which the Bromsulphalein was injected into the venous blood, the calculated plasma disappearance rates were determined after mixing was complete, and were comparable to the PDR of Ingelfinger and his associates.

RESULTS

Selective perfusion through the hepatic artery or the portal vein did not demonstrate a significant difference between the plasma Bromsulphalein disappearance rates. The data from 28 separate Bromsulphalein experiments in 5 different isolated liver preparations are summarized in Table I. The mean dye disappearance rate for hepatic artery perfusion alone was 64 per cent per minute (range 52 to 81); for portal vein perfusion alone 70 per cent per minute (range 52 to 91); and for the combined perfusion of both vessels it was 81 per cent per minute (range 63 to 91).

Duplicate determinations of dye disappearance in the same isolated liver did not demonstrate a preferential excretory pathway. In a representative experiment (Fig. 1) either the hepatic artery or the portal vein alone was perfused at a rate of 102 ml. per minute, or both vessels were perfused at a total flow rate of 204 ml. per minute. All BSP studies were conducted far enough

*(1) $C_2 = C_1 (d)^{t_2 - t_1}$

(2) $(t_2 - t_1)(\text{Log } d) = \log \frac{C_2}{C_1}$

(3) $1 - d = \text{PDR}$

Where:

C_1 = concentration of BSP at time t_1

C_2 = concentration of BSP at time t_2

PDR = plasma disappearance rate

apart to prevent overlapping of successive clearance determinations. A semilog plot of the second group of injections in the same study (Fig. 2) shows graphic calculations of the plasma disappearance rates from the straight line portion of the plasma dye disappearance curves.

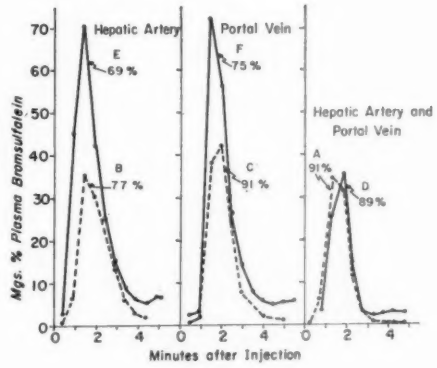

Fig. 1.

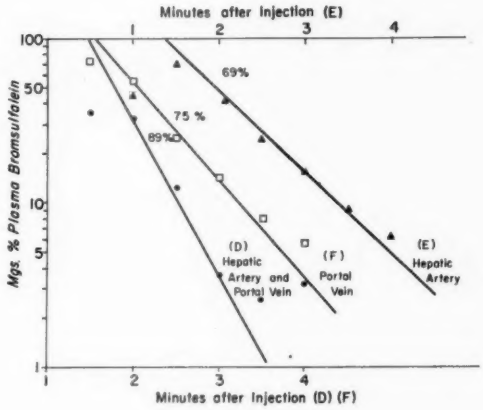

Fig. 2.

Fig. 1.—Plasma BSP disappearance rates in isolated liver (Experiment 5). Letters refer to the order in which the injections were made. Flow rates through individual vessels were equal.

Fig. 2.—Plasma BSP disappearance rates in isolated liver (Experiment 5). Semilog plots of clearance determinations *D*, *E*, and *F* from Fig. 1.

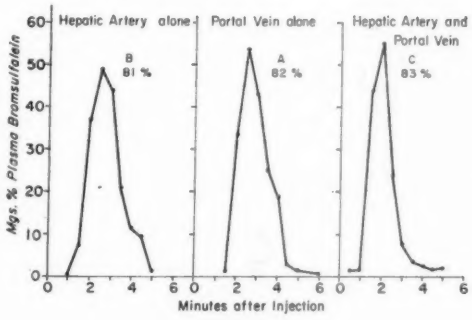

Fig. 3.

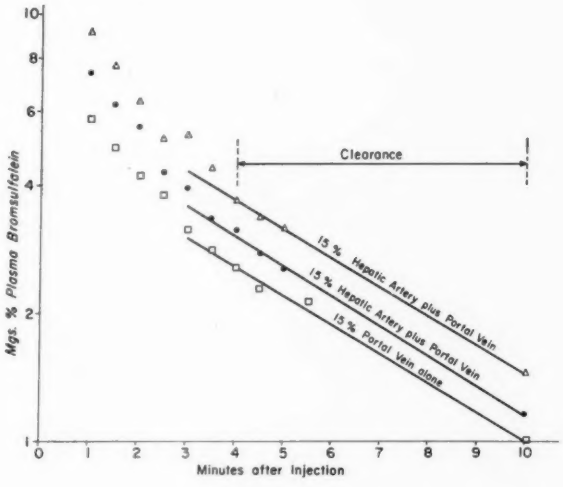
Clearance
15 % Hepatic Artery plus Portal Vein
15 % Hepatic Artery plus Portal Vein
15 % Portal Vein alone
Mgs. % Plasma Bromsulfalein
Minutes after Injection

Fig. 4.

Fig. 3.—Plasma BSP disappearance rates in isolated liver (Experiment 4). Clearances are nearly identical when perfused through alternate circulatory routes.

Fig. 4.—Plasma BSP disappearance rates in "intact" calf (Experiment 9). Clearances were determined between 4 and 10 minutes, after mixing was completed, and are identical.

In another representative study, the plasma Bromsulphalein disappearance rates were nearly identical, regardless of the route of blood supply to the liver (Fig. 3).

In the intact animals the plasma Bromsulphalein disappearance rates were identical regardless of the source of blood supply to the liver. Interrupting blood flow in the hepatic artery had no effect upon the clearance of BSP by the liver (Fig. 4).

TABLE I. PLASMA BROMSULPHALEIN DISAPPEARANCE RATES

EXPERIMENTAL CONDITIONS	TIME OF BSP INJECTION (HOURS AFTER START OF PERFUSION)	BSP INJECTED (MG. BSP/GM. LIVER)	VESSELS PERFUSED					
			PORTAL VEIN		HEPATIC ARTERY		PORTAL VEIN AND HEPATIC ARTERY	
			FLOW RATE (ML./MIN.)	DISAPPEARANCE (%/MIN.)	FLOW RATE (ML./MIN.)	DISAPPEARANCE (%/MIN.)	FLOW RATE (ML./MIN.)	DISAPPEARANCE (%/MIN.)
Isolated perfused liver	1	0.24	87	82	0	—		
	2	0.24	0	—	87	55		
	4¼	0.22	162	62	0	—		
BSP injected into inflow vessel(s)	6	0.24	0	—	180	52		
	1½	0.24	100	48	100	—		
	3¼	0.31	0	—	100	64		
	5	0.32	200	68	0	—		
	7	0.31	0	—	200	65		
	10	0.33	—	—	—	—	430	63
	1	0.23	100	74	0	—		
	2	0.20	0	—	100	53		
	4	0.29	215	52	0	—		
	6	0.36	0	—	215	60		
	½	0.19	88	82	0	—		
	2	0.18	0	—	85	81		
	3½	0.19	—	—	—	—	173	83
	1	0.14	—	—	—	—	204	91
	2½	0.12	0	—	102	77		
	3½	0.10	102	91	0	—		
	6	0.13	—	—	—	—	204	89
	7	0.13	0	—	102	69		
	8	0.10	102	75	0	—		
Isolated perfused liver	1½	0.09					190	11
	2½	0.08					190	15
	3½	0.09					190	26
BSP injected into venous return from liver to oxygenator	2	0.10					190	12
	3	0.09					190	18
	4	0.10					190	23
	(hours after start of operation)	Total BSP injected (mg.)						
Intact animal	2	202	—	18				—
	4	222	—	—				15
BSP injected into peripheral vein	1½	155		—				13
	2½	160		—				15
	3½	157		18				—

In the "intact" animal and in the isolated organ, the pattern of appearance of BSP in the bile after uptake from the blood was similar. In either case the dye was secreted into the bile in high concentrations (Figs. 5 and 6).

When the Bromsulphalein was injected directly into the hepatic artery or the portal vein, factors which determined the turnover rate of the total blood pool (that is, total flow rate, total blood volume) were not observed to influence the plasma BSP disappearance rate. In contrast, these factors did influence the dye disappearance rate when the dye was injected into the venous return from

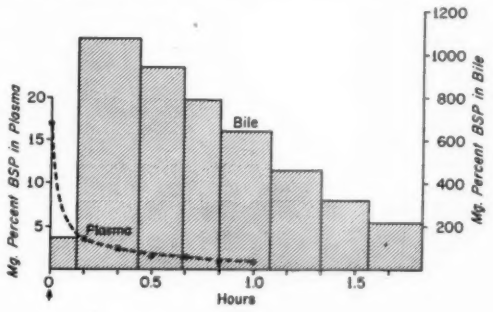

Fig. 5.

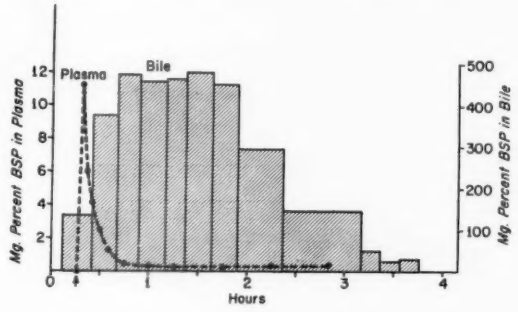

Fig. 6.

Fig. 5.—Plasma BSP disappearance following a single injection in an "intact" animal, correlated with the appearance of the dye in bile.

Fig. 6.—Plasma BSP dye disappearance following a single injection in an isolated perfused liver, correlated with the appearance of the dye in the bile.

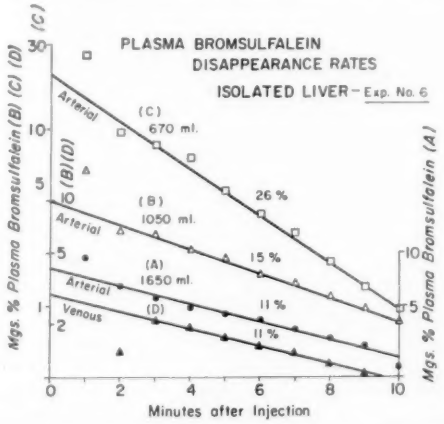

Fig. 7.—Plasma BSP disappearance rates in isolated liver (Experiment 6). Clearance of BSP is related to the estimated reservoir volume (includes circulation tubing) of the system and the total flow rate. A shorter turnover time produces a higher clearance rate of Bromsulphalein from the plasma.

the liver and allowed to mix with the reservoir pool before being presented to the liver. These important points are illustrated in Fig. 7. As the estimated reservoir volume* was varied from 1,650 ml. to 670 ml. while the same total flow rate was maintained, the experiment with the lowest perfusate volume had the highest plasma BSP disappearance rate in spite of the increased concentration of plasma Bromsulphalein.

DISCUSSION

Substances that appear in bile have been divided into 3 groups on the basis of the degree of concentration or dilution they exhibit during passage from

*Includes reservoir volume plus mechanical volume of apparatus.

blood to bile.[6] One group (A) appears in bile in the same concentration as in blood. This includes Na^+, Cl^-, K^+, and glucose. A second group (B) appears in bile concentrated as much as 100 times and includes the bile salts, bile pigments, rose bengal, fluorescein, and Bromsulphalein. The third group (C) is retained in the blood and appears in the bile only in very low concentrations (if at all). This includes alkaline phosphatase, inulin, sucrose, phospholipids, and mucoproteins.

Studies of bile secretion by Cook and colleagues[12] demonstrated low and comparable rates of clearance into bile for substances in the unconcentrated group (A). The bile-blood concentration ratios approached unity and their clearances were increased by hydrocholeretics.[13] These investigators concluded that substances in the unconcentrated group were excreted by a process suggestive of filtration.

In the isolated perfused rat liver, relationships between bile flow, bile secretory pressure, and perfusion pressure delivered to the liver would seem to rule out filtration as a major mechanism of bile formation.[8] Maximum bile secretion pressures always exceeded perfusion pressures by 3 to 9 cm., and bile flow was independent of blood flow at physiologic perfusion pressures.

Bile flow is dependent upon maintenance of normal temperatures and decreases rapidly with decreasing temperature.[7, 8] This is in contrast to the kidney in which urine production is primarily dependent upon a filtered solute load. A reduction of temperature and consequent reduction of tubular resorption activity is accompanied by an increase in urine flow.[22]

The substances of the concentrated group (B) appear in bile in such high concentrations that an active secretory process must be involved in their transfer from blood to bile.[6, 12, 23] As a representative of this group, Bromsulphalein has been the most extensively studied. There is a limiting rate of dye secretion beyond which increases in plasma BSP concentration no longer result in an increase in the bile secretory rate of BSP.[21] This saturation phenomenon appears to be characteristic of Class B compounds.[12] The transfer of BSP from blood to bile involves a storage phase[9] and it is excreted into the bile primarily as amino acid conjugates.[15, 19, 20] After the initial mixing period following intravenous injection, a significant amount of the plasma BSP is in the conjugated form, indicating re-entry into the circulation after its original passage into the hepatic cell.[17] A small percentage of these BSP metabolites are colorless and account for some of the discrepancies between injected and recovered S^{35}-labeled BSP.[9]

Recently the interesting view has been advanced that the blood-bile transfer of BSP and other important bile substances does not occur across the hepatic parenchymal cells but from or within the bile duct cells[1] supplied by the peribiliary arterial plexus.[3]

From experiments conducted in isolated perfused canine livers, Andrews and colleagues,[2, 4] demonstrated greater removal of BSP from blood perfused through the hepatic artery than from similar flows of blood perfused through

the portal vein. The bile ducts were depicted as structures analogous to the renal tubules and intrahepatic cholestatic jaundice would then be similar to anuria.

With simultaneous injection of nonlabeled BSP into the portal vein and S^{35}-labeled BSP into the hepatic artery of dogs, Brauer[5, 10] did not demonstrate preferential excretion of hepatic artery BSP into the bile. These investigators found that BSP injected into the portal vein was actually excreted into the bile in higher concentrations than the BSP injected into the hepatic artery. It should be emphasized that, in these experiments, the secretion of BSP into bile was studied and not the removal of BSP from the blood by the different circulations. Different processes possessing different reaction rates may be involved in hepatic uptake of BSP from the blood, transport through the parenchymal cell, and excretion of BSP and its metabolic products into the bile.

Additional support that the hepatic parenchymal cell is the principal site of dye excretion comes from visual observation of fluorescein excretion with intravital fluorescence microscopy. Hanzon[16] has described a sequence of uptake into the parenchymal cells, secretion into the bile canaliculi, and transport down the biliary tree for this substance.

The studies of BSP clearance reported here have not documented a preferential arterial route of BSP uptake in the bovine liver. All experiments, both in isolated livers and in intact animals showed no significant differences in the clearance of BSP when blood was supplied to the liver through alternate routes. The high concentration of BSP observed in the bile in all species studied,[19] including the calf, is evidence for an active secretory process regulating the excretion of this dye, but the actual mechanisms of bile formation cannot be determined definitely on the basis of the available evidence. These experiments with bovine liver are interpreted as indirect support that the hepatic parenchymal cells serve as the major site of bile formation.

CONCLUSIONS

Studies of Bromsulphalein clearance were conducted in a reproducible, apparently bacterially sterile, isolated perfused bovine liver. Both venous and arterial hepatic circulations contribute to the excretion of Bromsulphalein, and a preferential clearance of this dye by the hepatic arterial circulation was not demonstrated.

Bromsulphalein clearance studies in intact animals provided similar results.

Although species differences may be involved, these experiments do not support the concept that the bile duct cells supplied by the peribiliary arterial plexus are the principal or exclusive sites of removal of BSP from the blood; instead, this function probably resides mainly in the hepatic parenchymal cells.

In the isolated perfused calf liver, the clearance of BSP is directly related to the factors which determine the turnover rate of total blood pool.

REFERENCES

1. Andrews, W. H. H.: Excretory Function of the Liver, Lancet **269**: 166-169, 1955.
2. Andrews, W. H. H., Maegraith, B. G., and Richards, T. G.: Effect Upon Bromsulphalein Extraction Rate and Distribution of Blood Flow in Perfused Canine Liver, J. Physiol. **131**: 669-677, 1956.

3. Andrews, W. H. H., Maegraith, G. C., and Wenyon, C. E. M.: Studies on Liver Circulation: Micro-anatomy of Hepatic Circulation, Ann. Trop. Med. **43**: 229-237, 1949.
4. Andrews, W. H. H., and Richards, T. G.: Removal of BSP by the Portal Vein and the Hepatic Artery in the Anesthetized Dog, J. Physiol. **131**: 21-22, 1956.
5. Brauer, R. W., editor: Liver Function Symposium on Approaches to Quantitative Description of Liver Function, American Institute of Biological Science, Pub. 4, 1958, p. 248.
6. Brauer, R. W.: Mechanisms of Bile Secretion, J. A. M. A. **169**: 1462-1466, 1959.
7. Brauer, R. W.: Physiology of Induced Hypothermia *in* Dripps, R. D., editor: Proceedings of Symposium, Oct. 28-29, 1955, Washington, D. C., 1956, Division of Medical Sciences, National Academy of Sciences, National Research Council, p. 235.
8. Brauer, R. W., Leong, G. F., and Holloway, R. J.: Mechanics of Bile Secretion: Effect of Perfusion Pressure and Temperature on Bile Flow and Bile Secretion Pressure, Am. J. Physiol. **177**: 103-112, 1954.
9. Brauer, R. W., Pessotti, R. L., and Krebs, J. S.: Distribution and Excretion of S^{35}-Labeled Sulfobromophthalein Sodium Administered to Dogs by Continuous Infusion, J. Clin. Invest. **34**: 35-43, 1955.
10. Brauer, R. W., Shill, O. S., and Krebs, J. S.: Dependence of Biliary BSP Excretion on Route of Administration (Hepatic Artery or Portal Vein), Fed. Proc. **16**: 284-285, 1955.
11. Chapman, N. D., Goldsworthy, P. D., Martinis, A. J., Volwiler, W., and Nyhus, L. M.: Techniques of Isolated Liver Perfusion. In preparation.
12. Cook, D. L., Lawler, C. A., Calvin, L. D., and Green, D. M.: Mechanisms of Bile Formation, Am. J. Physiol. **171**: 62-74, 1952.
13. Cook, D. L., Lawler, C. A., and Green, D. M.: Studies on the Effect of Hydrocholeretic Agents on Hepatic Excretory Mechanisms, J. Pharmacol. & Exper. Therap. **110**: 293-299, 1954.
14. Gaebler, O. H.: Determination of Bromsulphalein in Normal, Turbid, Hemolized, or Acteric Serums, Am. J. Clin. Path. **15**: 452-455, 1945.
15. Grodsky, G. M., Carbone, J. V., and Fanska, R.: Metabolism of Sulphobromophthalein, Nature **183**: 469-470, 1959.
16. Hanzon, V.: *in* Brauer, R. W., editor: Liver Function Symposium on Approaches to Quantitative Description of Liver Function, American Institute of Biological Science, Pub. 4, 1958, p. 281.
17. Higgins, F. E., Foulk, W. T., and Bollman, J. L.: Column Chromotography of Bile, Serum, and Urine After Intravenous Administration of Sulfobromophthalein, Gastroenterology **38**: 194-202, 1960.
18. Ingelfinger, F. J., Bradley, S. J., Mendeloff, A. I., and Kramer, P.: Studies With Bromsulfalein, I. Its Disappearance From the Blood After a Single Intravenous Injection, Gastroenterology **11**: 646-657, 1948.
19. Krebs, J. S.: *in* Brauer, R. W., editor: Liver Function Symposium on Approaches to Quantitative Description of Liver Function, American Institute of Biological Sciences, Pub. 4, 1958, p. 302.
20. Krebs, J. S., and Brauer, R. W.: Metabolism of Sulfobromophthalein Sodium (BSP) in Rat, Am. J. Physiol. **194**: 37-43, 1958.
21. Mendeloff, A. I., Kramer, P., Ingelfinger, F. J., and Bradley, S. J.: Studies With Bromsulfalein, II. Factors Altering Its Disappearance From the Blood After a Single Intravenous Injection, Gastroenterology **13**: 222-234, 1949.
22. Moyer, J. H., Morris, G. C., and De Bakey, M. E.: Physiology of Induced Hypothermia, *in* Dripps, R. D., editor: Proceedings of Symposium, Oct. 28-29, 1955, Washington D. C., 1956, Division of Medical Sciences, National Academy of Sciences, National Research Council, p. 199.
23. Popper, H., and Schaffner, F.: Liver: Structure and Function, New York, 1957, The McGraw-Hill Book Co., Inc., p. 85.

(For discussion, see page 152.)

STUDIES ON PORTAL HYPERTENSION

V. A Comparison Between the Experimentally Induced State of Portal Hypertension and That Observed in Human Beings

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WITH the introduction of the experimental method in medicine, reproduction in the laboratory animal of disease processes observed in human beings became an essential part of medical research. Animal experimentation, although limited in clinical application by differences in species, has been responsible for much of our present knowledge of pathologic deviation from normal function.

However, there are certain pathologic states which have proved particularly refractory to reproduction in the experimental animal, and thus it has become almost axiomatic to accept such processes as unique to man. The state of portal hypertension associated with many cases of cirrhosis of the liver and with extrahepatic obstructions of the portal system has been, perhaps, one of the most commonly used examples of an unreproducible state. Taylor,[19] as recently as 1957, reaffirmed this view in a critical analysis of experimental work in this area.

Efforts to reproduce the state of portal hypertension in the laboratory animal have been oriented in several directions and the methods utilized have been classified as intrahepatic, extrahepatic, or as a combination of both.[21] In the first category, the injection of foreign material into the portal vein or into the liver substance, the imposition of deficiency diets, and the exposure to various hepatoxic agents are among the more commonly utilized procedures. In the second group, partial and/or progressive constriction of the splenic, portal, or hepatic veins and the creation of arteriovenous fistulas have been used. Although a certain degree of success followed the repeated injection of silicon dioxide[16] or the application of ingenious devices to impair the flow of blood in the hepatic veins,[21] the general opinion prevails that the syndrome of portal hypertension is not fully reproducible in the experimental animal. It has been stated that the enigma of portal hypertension will remain unsolved until its experimental reproduction is obtained,[19] that an erect posture is necessary for the development of dilated esophageal veins,[13] and also that the large number of collateral pathways available in the experimental animal generally prevents the formation of esophageal varices.[6] Nevertheless, despite these conclusions, rats made cirrhotic by carbon tetrachloride have been shown to exhibit many of the

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hemodynamic situations associated with the human state of portal hypertension[2, 8] and more recently, the regular formation of esophageal varices has been demonstrated in mice infected with *Schistosoma mansoni*.[20]

The purpose of this paper is to report the results of an experimental study in which, it is believed, a complete state of portal hypertension has developed in a large colony of cirrhotic rats. By using diagnostic methods akin to those utilized in an equally large series of human patients with portal hypertension, a comparison between the two situations was made possible and the similarity in the basic mechanisms of the hemodynamic response appeared so close as to make them practically indistinguishable. This report is part of a series relating to a comprehensive study of the normal and pathologic physiology of the portal circulation being conducted by this group.[9, 10, 11, 14]

MATERIALS AND METHODS

Two hundred and thirty-nine albino rats of the Wistar strain constituted the material for the experimental phase of this study. A series of 318 patients in whom an evaluation of the portal circulation was performed was used for purposes of comparison study. Part of this last series has been previously reported.[14] The rats were adult male animals with an average weight of 350 grams and were fed standard Purina rat stock diet and water ad libitum. Carbon tetrachloride, at a dosage of 0.1 ml. per 100 grams of body weight diluted in 3 parts of liquid petrolatum, was administered by direct instillation into the stomach via an oral gastric tube. This dosage was maintained at a rate of twice weekly for an initial 10 doses and was then reduced to once weekly for a period of 3 months in 50 per cent of the animals, of 4 months in 20 per cent of the animals, and of 6 months in another 20 per cent of the animals. Ten per cent of the animals were not exposed to the toxic agent and served as a control group. Once the designated period of exposure was completed on each group of animals, carbon tetrachloride administration was interrupted in approximately 45 per cent of the group for the evaluation of any improvement that might follow this interruption of the toxic agent for a period of 3 months. The remainder of the animals were maintained on the same dosage to the conclusion of the experiment which also lasted an additional 3 months.

Exploratory laparotomies were performed periodically, vascular changes were observed, and liver biopsies obtained. At the time of sacrifice a careful dissection of the portal venous bed and tributaries was performed with and without the injection of colored plastic materials. Information obtained by these last methods was used to confirm radiologic findings but was considered unsatisfactory in comparison to the in vivo demonstration of the normal or altered hemodynamics of the portal circulation. Accordingly, more emphasis was placed on the findings provided by serial angiography in the living subject which offered a dynamic rather than a static demonstration of the circulatory disturbances present in these animals.

An adaptation of the technique for obtaining multiple-exposure serial splenoportograms in human beings was developed for use in the rat. Although the original technique of splenoportography was developed in the dog[1] and excellent use of the method in this animal has been published,[5] no reports could be found on the utilization of splenoportography in an animal as small as the rat. With the use of light ether anesthesia a small incision was made on the left side of the abdomen and the spleen was exposed. A No. 22 gauge needle attached to a 10 ml. syringe by a length of polyethylene tubing was inserted into the spleen and 1 ml. of radiopaque dye (Hypaque 50 per cent) was deposited without pressure into the splenic pulp. The course of the blood, mixed with the radiopaque dye from the splenic pool, was then followed by a rapid sequence of x-ray films. Starting at the initiation of the injection, 15 films were exposed at the rate of 1 film every one-half second for the first 10 exposures and of 1 film per second thereafter. In the early part of the investigation, a Fairchild Odelca, 70 mm. roll film camera was used, but because of the

small size of the film and its considerable grainy quality it was regarded as unsatisfactory. The majority of the splenoportograms were then taken with a Fairchild x-ray camera with an F-280 roll film cassette. The average exposure factors for a rat of the size utilized in this experiment was 200 Ma., 1/20 second, 68 kv., at a target film distance of 40 inches. With the larger sized films provided by this equipment the entire body of the animal could be included and good detail was preserved. Splenoporotgrams were obtained in randomized members of each group of animals, including the control group, to reach a total number of 171 multiple-exposure serial splenoportograms. These serial splenoportograms were repeated for a second time in over one half of the animals and for a third and fourth time in particularly interesting members of the colony.

A simple water manometer as well as a strain gauge and an electromanometer were used without success in an attempt to determine the pressure in the splenic pulp. It was decided that, at least under the conditions of this experiment, valid manometric splenic pulp readings were not obtainable in the small spleen of the rat. Therefore pressure in the portal vein was determined by direct cannulation through the superior mesenteric vein with a No. 18 gauge needle. With a needle of this size, capillary phenomena known to influence manometric readings were avoided. However, trauma to the venous wall made the sacrifice of the animal necessary, and for this reason manometric readings were not taken in all animals but in randomized members of each group.

A micromethod for the determination of Bromsulphalein retention[12] was adapted for use in the rat and by this means consistent results could be obtained from the limited amounts of blood obtained by the sectioning of a small portion of the tail. Fifty milligrams of Bromsulphalein per kilogram of body weight was used instead of the 5 mg. dosage described in the original method.

The studies performed in the series of human patients with portal hypertension have been reported elsewhere.[10, 14]

RESULTS

Because of the acute toxic effect of the initial biweekly gastric instillation of carbon tetrachloride, an unusually high mortality rate was experienced in the early part of the experiment. When the administration of the toxic agent was reduced to once weekly, the rate diminished considerably. However, death due to these toxic effects, to trauma associated with handling of the animals during repeated insertions of the gastric tube, and to the usual spontaneous diseases affecting these animals, reduced the colony by 107 members. Thus, 104 animals exposed to carbon tetrachloride and 28 controls survived for useful purposes in this experiment.

Sensitivity to carbon tetrachloride in terms of the development of cirrhotic changes varied considerably in different members of the colony. In general, the degree of hepatic cirrhosis obtained appeared to depend more on the individual sensitivity of the animal than on the time of exposure to the toxic agent. Again, in a general way, variations of severity of cirrhotic involvement in the group exposed for 3 months did not differ to a great extent from the changes observed in the groups exposed for 4 and 6 months. Mild and severe degrees of cirrhosis were observed but in slightly different proportions, in all groups of exposed animals. For the purposes of this report the extent of cirrhotic changes will be classified as mild and severe irrespective of the minor variations associated with the time of exposure to the toxic agent. Changes in Bromosulphalein retention in general followed the histologic changes.*

*Changes in liver function tests will be reported in a subsequent article.

Of the 104 animals which survived the exposure to carbon tetrachloride, 81 were submitted to serial splenic portography. Of these, 42 underwent a second splenoportogram at a subsequent time, 9 a third splenoportogram, and 1 a fourth splenoportogram. Of the 28 members of the control group, 23

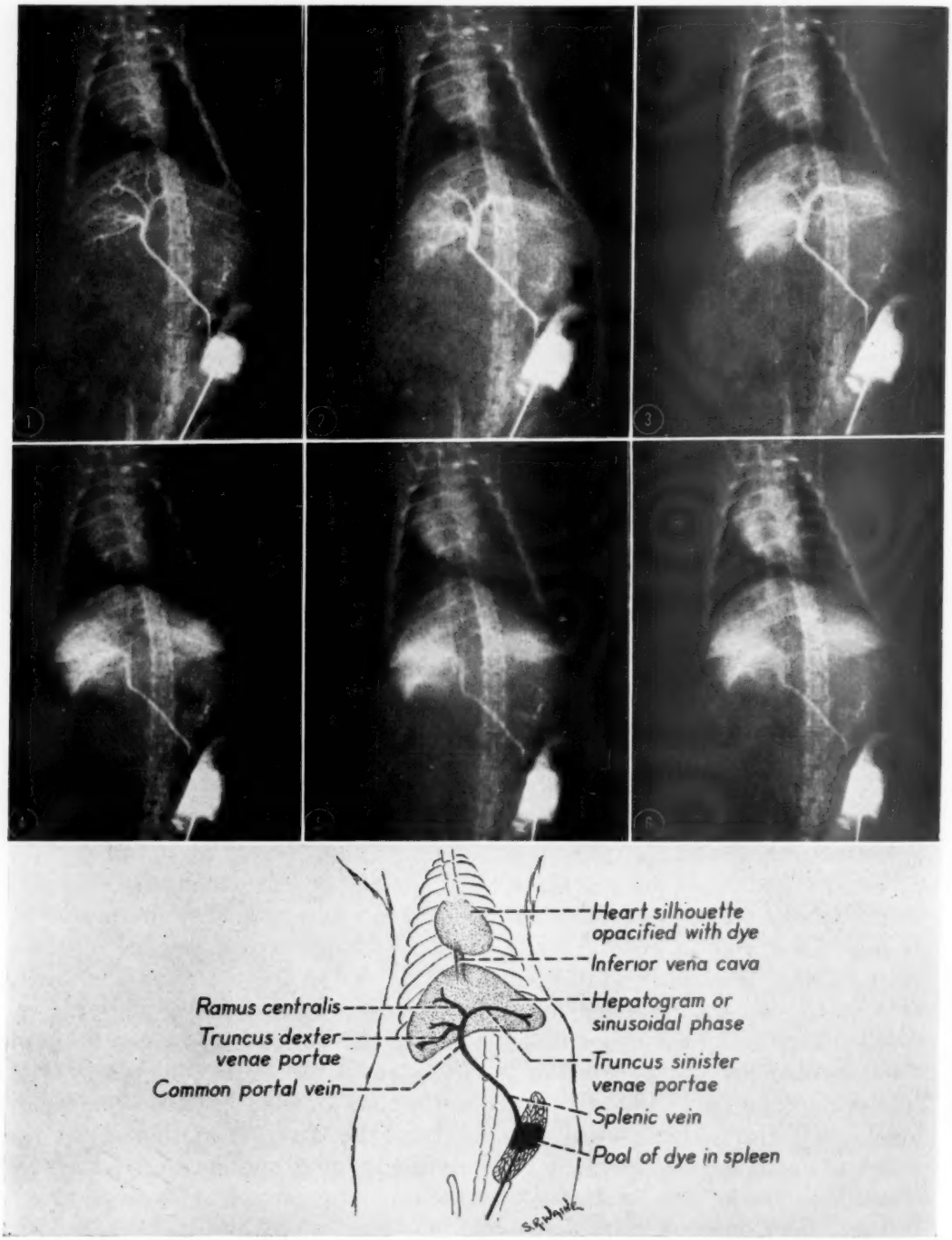

Fig. 1.—Six films selected from a 15-film, multiple-exposure splenoportogram in a normal rat. The dye has been deposited in the splenic pulp without pressure. The normal venous components of the portal system are indicated in the line drawing.

underwent 1 portogram and 4 a second portogram. A representative splenoportogram in the control group is depicted in Fig. 1. The anatomic arrangement of the main branches of the portal vein can be compared favorably with the studies of Gershbein and Elias.[4] In Fig. 2 the normal radiologic appearance of the inferior vena cava of the rat is demonstrated both by direct injection of the left iliac vein and of the left renal vein. These normal roentgenograms were obtained to serve as orientation in the identification of these vessels when they were filled through abnormal portal systemic communications as shown by splenoportograms of cirrhotic animals with portal hypertension.

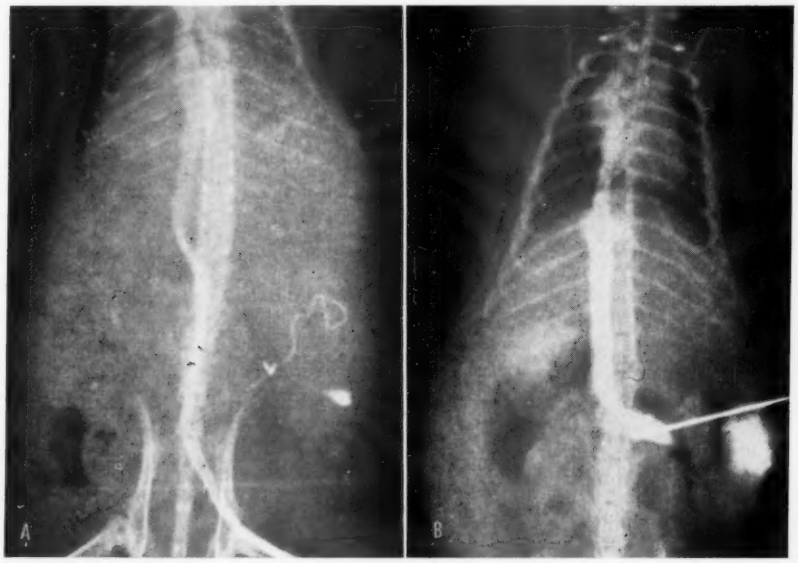

Fig. 2.—The normal radiologic appearance of the inferior vena cava of the rat for purposes of orientation and comparison is demonstrated by direct injection of the left iliac and left renal veins. The middle part of the normal S-shaped contour corresponds to the confluence of the left renal vein.

In the 81 cirrhotic animals in which splenoportograms were performed, 64 showed angiographic abnormalities in portal hemodynamics while 17 had essentially normal findings. A similar incidence of normal-appearing splenic portograms was observed in the series of human patients with cirrhosis of the liver. Besides the more common abnormalities observed in the splenoportograms of the cirrhotic rats (distortion and dislocation of intrahepatic branches of the portal vein and irregularities of sinusoidal filling in the hepatogram phase present, respectively, in 89.1 per cent and 75 per cent of the cases), the more striking finding regularly noted was the marked tendency of the portal blood to bypass the cirrhotic liver and reach the heart via vessels of the systemic circulation. This hepatic bypass was effected through pathways almost identical with those observed in the series of human patients[14] (Fig. 3). Portal blood was observed to reach the systemic circulation either by reversing its direction of flow into already functioning normal components of the portal system or by the establishment of larger portal systemic communications (natural shunts) frequently mediated through reopened embryonic channels. As was observed in human beings with portal hypertension, reversal of flow into veins of the portal system occurred mainly

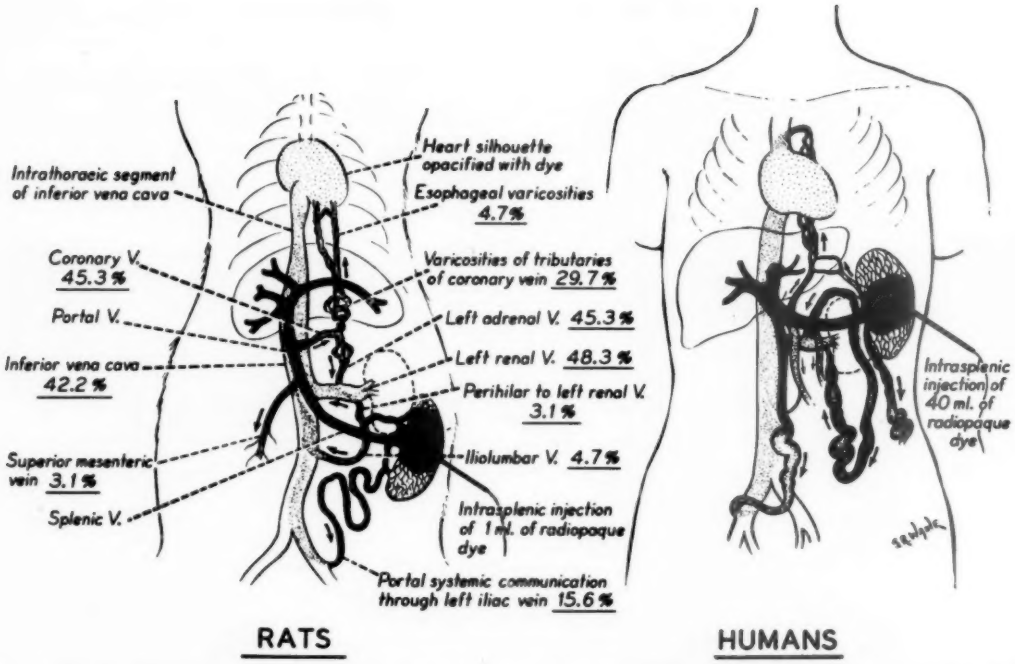
Intrathoraeic segment of inferior vena cava
Coronary V. 45.3 %
Portal V.
Inferior vena cava 42.2 %
Superior mesenteric vein 3.1 %
Splenic V.
Heart silhouette opacified with dye
Esophageal varicosities 4.7%
Varicosities of tributaries of coronary vein 29.7 %
Left adrenal V. 45.3 %
Left renal V. 48.3 %
Perihilar to left renal V. 3.1 %
Iliolumbar V. 4.7 %
Intrasplenic injection of 1 ml. of radiopaque dye
Portal systemic communication through left iliac vein 15.6 %
Intrasplenic injection of 40 ml. of radiopaque dye
RATS
HUMANS

Fig. 3.—Diagrammatic presentation of the main routes of portal diversion as observed in the experimental animal and in human patients with portal hypertension.

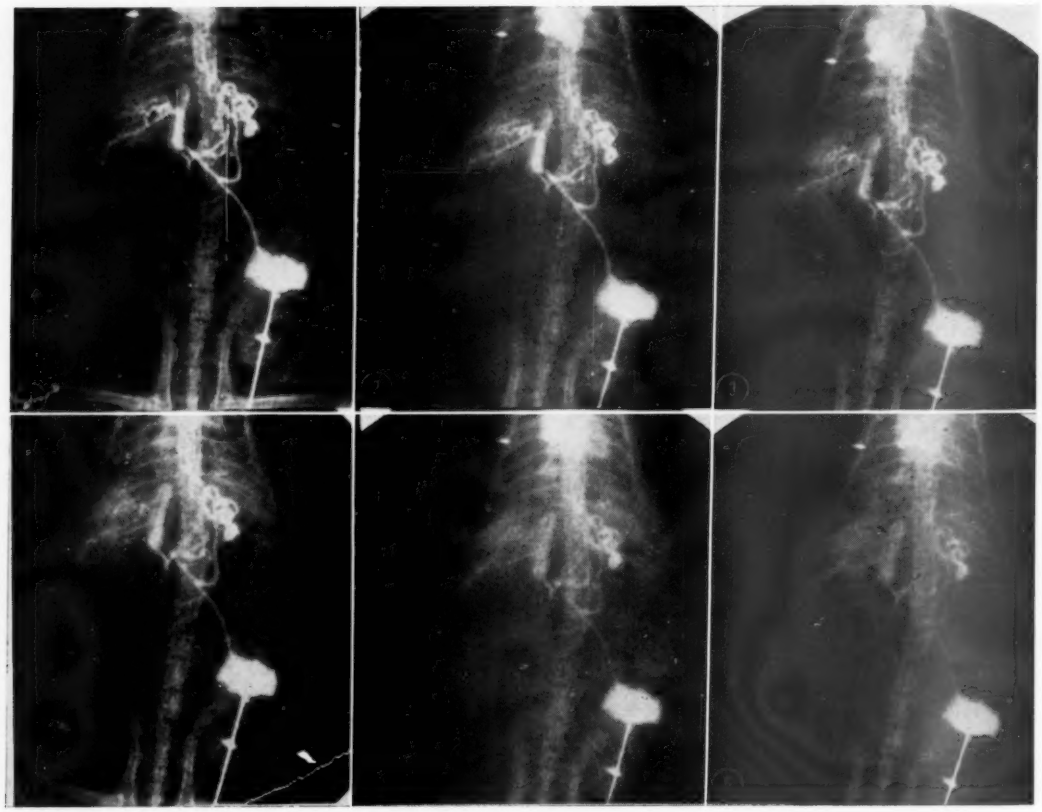

Fig. 4.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Varicosities in the area of the cardia of the stomach and along the esophagus are filled by reverse flow into the coronary vein. The portal vein is markedly dilated and there are distorted intrahepatic branches. Sinusoidal filling is diminished and there is an incomplete hepatogram phase.

through dilated tributaries of the coronary vein around the cardia of the stomach and through distended, tortuous esophageal veins (Figs. 4-7). To a lesser degree this backflow was observed to occur in the mesenteric vein (Table I). Also as was observed in human beings, bypass through larger connecting channels took place through reopened embryonic vessels as was the case in the animals in which the umbilical vein carried portal blood into the superficial abdominothoracic veins to sometimes form the classic *caput medusa* arrangement (Figs. 8 and 9). In other instances these portal systemic connections were established through the left renal vein. Portal blood reached the left renal vein through communications between the retrogastric plexuses and the periadrenal plexuses with the adrenal vein finally carrying the portal blood into the renal vein (Fig. 10). This last was a frequently observed type of communication (Table I) and its course was confirmed by in vivo dissec-

TABLE I. DIVERSION OF PORTAL BLOOD INTO THE SYSTEMIC CIRCULATION (HEPATIC BYPASS)

Backflow into veins of the portal system: 82.8%	Coronary vein	45.3%
	Varicosities in cardia of stomach	29.7%
	Varicosities in esophageal veins	4.7%
	Mesenteric vein	3.1%
Diversion through more direct portasystemic "natural shunts": 78.1%	Adrenal vein into left renal vein	45.3%
	Hilar splenic branch into left iliac vein	15.6%
	Hilar splenic branch into iliolumbar veins	4.7%
	Hilar splenic branch into left renal vein	3.1%
	Umbilical vein into superficial abdominothoracic veins	9.4%

In many instances several types of abnormalities were observed to coexist in the same animal.

tions (Fig. 11) and by postmortem injection of colored plastic materials. In still other cases, the portal systemic bypass through "natural shunts" was accomplished by retroperitoneal veins connecting the hilum of the spleen with the left renal vein (Fig. 12), the hilum of the spleen with the iliolumbar vein (Fig. 13), and the hilum of the spleen with the left iliac vein (Figs. 14 and 15). Many of these situations were practically indistinguishable from those observed in human beings (Figs. 16-18). As a result of this active diversion of portal blood into the systemic circulation, the inferior vena cava was visualized in various extents in the splenoportograms of 42 per cent of the animals with abnormal changes.

The existence of these large natural shunts did not prevent the formation of esophageal varicosities, and this situation was comparable to the observations previously reported in man.[14] However, the one phenomenon not observed in the experimental animal was clinical bleeding into the gastrointestinal tract.

Manometric readings were taken in the portal vein cannulated through the mesenteric vein. Average portal pressure for the control group was 162.7 ± 5.6 mm. H_2O (Fig. 19 and Table II). In cases of mild cirrhosis the mean pressure of the group, 164.1 ± 7.8 mm. H_2O, was no different from that in the control group. Finally, in the group of animals with severe cirrhosis of the liver the mean pressure value was elevated to 216.7 ± 6.7 mm. H_2O. This difference was statistically highly significant, $P < 0.001$.

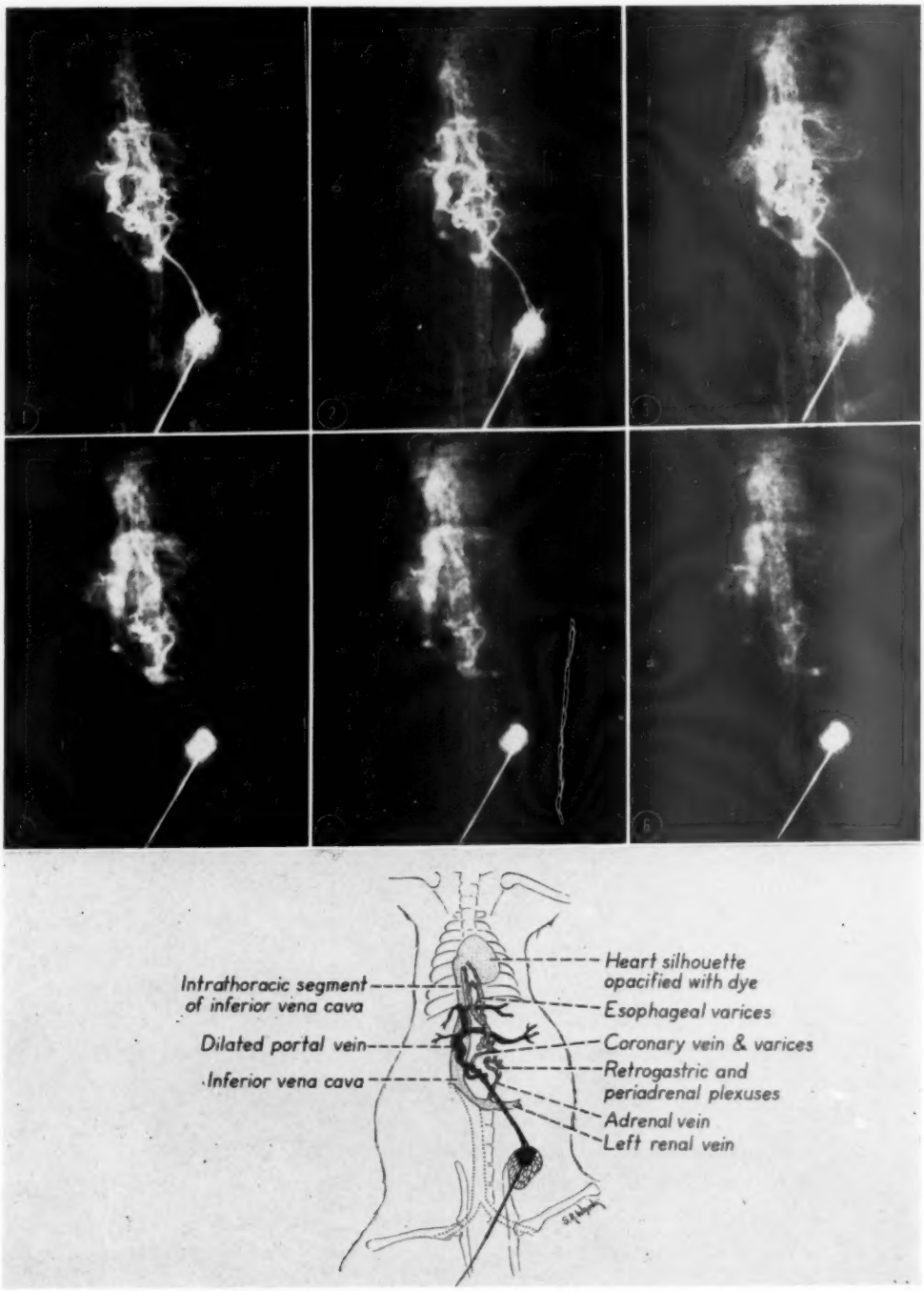

Fig. 5.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Splenic blood is carried by the splenic vein into a very distorted and dilated portal vein. The main branches of the portal vein are also markedly dilated. A coronary vein arises from the portal vein and empties into a convolution of dilated veins in the area of the lesser curvature of the stomach which in turn communicates with markedly dilated esophageal varicosities. From the retrogastric plexuses the left adrenal vein is filled with portal blood and empties into the left renal vein very near its junction with the inferior vena cava. In the 3 last films the splenoportal axis is not visualized any more whereas the coronary vein, the gastric and esophageal varicosities, and the adrenal vein still act as an active mechanism of hepatic bypass.

Fig. 6.

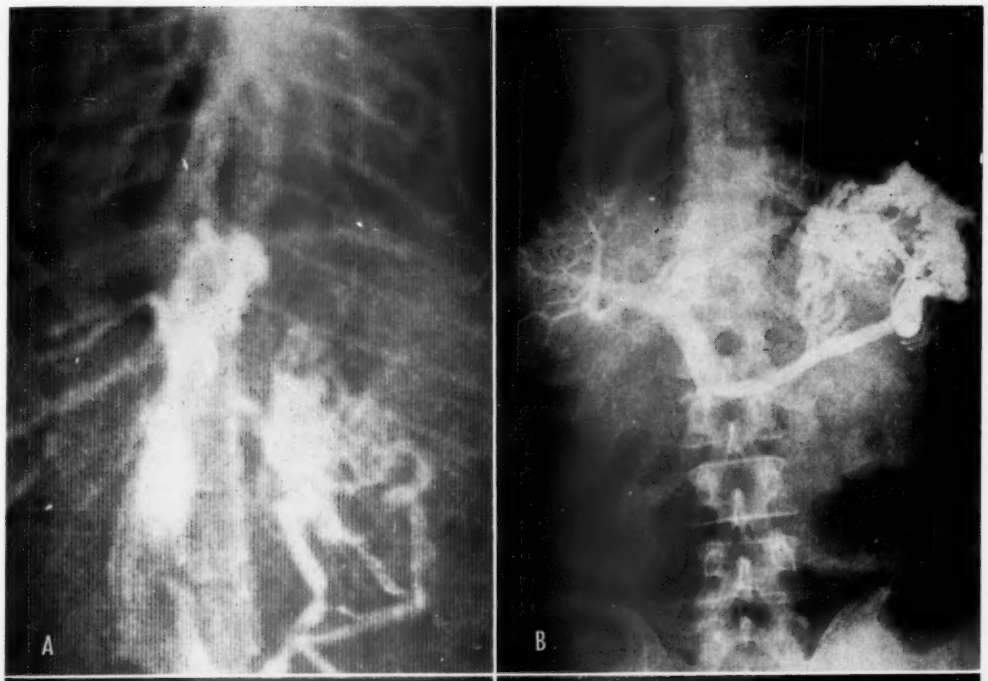

Fig. 7.

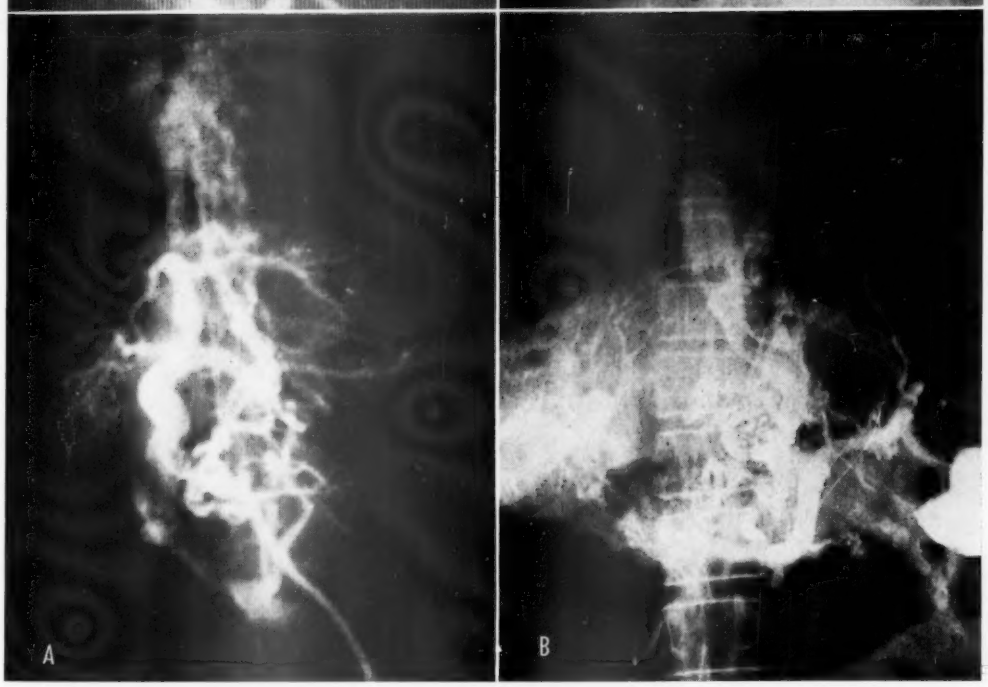

Fig. 6.—A comparison between the radiologic appearance of varicosities limited to the area of the cardia of the stomach. *A*, Film from a splenoportogram of a cirrhotic rat. *B*, Film from a splenoportogram of a human patient with portal hypertension.

Fig. 7.—A comparison between the radiologic appearance of gastroesophageal varicosities. *A*, Film from a splenoportogram of a cirrhotic rat. *B*, Film from a splenoportogram of a human patient with portal hypertension.

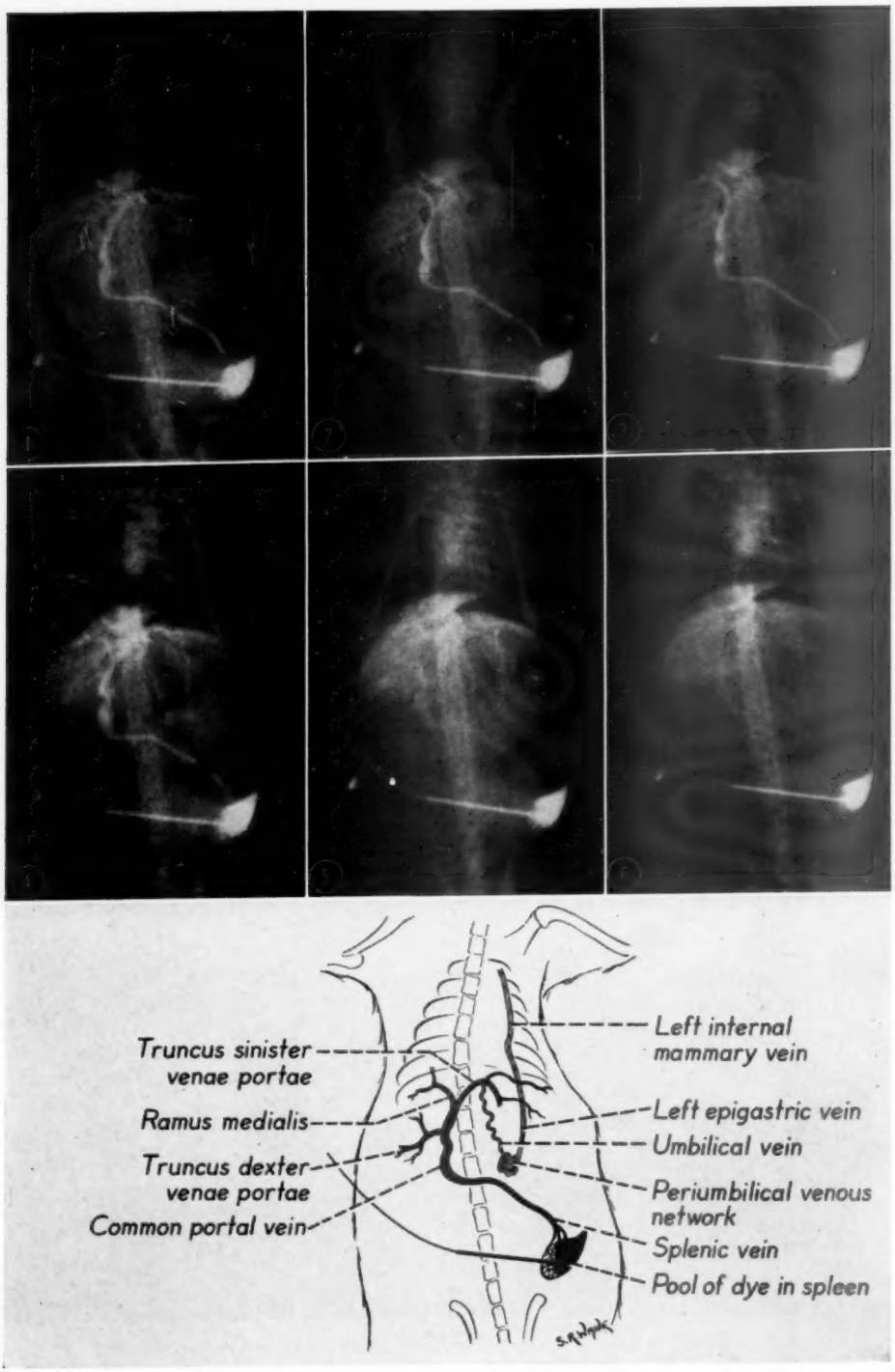

Fig. 8.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. A patent umbilical vein diverts portal blood from the left intrahepatic branch of the portal vein into a convolution of periumbilical vessels. From this area the left epigastric vein and left internal mammary vein are filled.

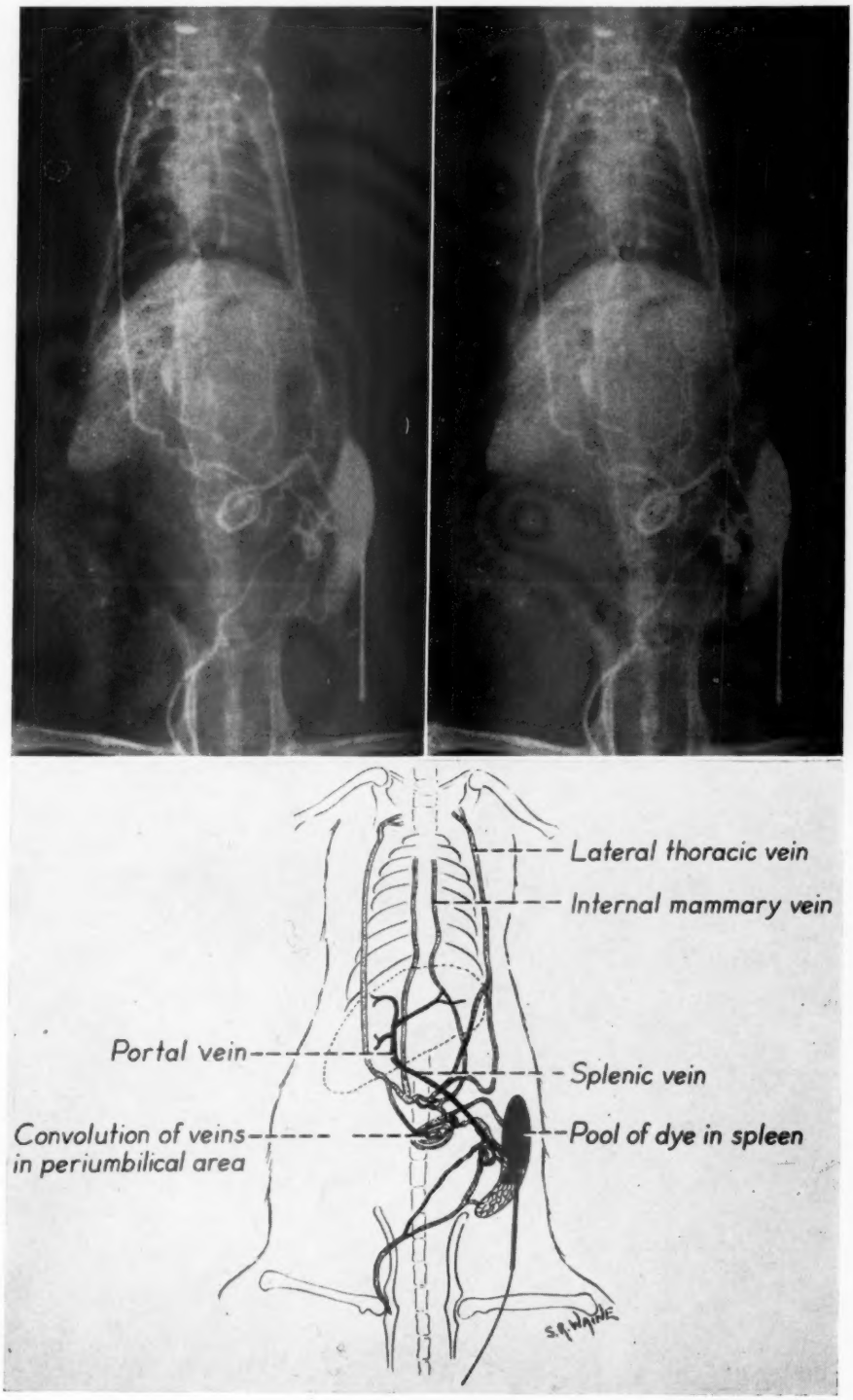

Fig. 9.—*Caput medusa* type of superficial abdominothoracic collateral circulation is depicted in these films from a splenoportogram of a cirrhotic rat.

The presence of ascites in varying degrees was observed in 59 per cent of the animals in the series.

Effect of Interruption of the Hepatotoxic Agent.—When the administration of the toxic agent was interrupted for a period of 90 days, which corresponds approximately to 9 years in the normal life span of man, improvement in the

Fig. 10.—This film is an enlargement of one of the exposures corresponding to the same animal depicted in Fig. 5. The bypass of portal blood is effected through a dilated left adrenal vein (1), into the left renal vein (2), and the vena cava (3).

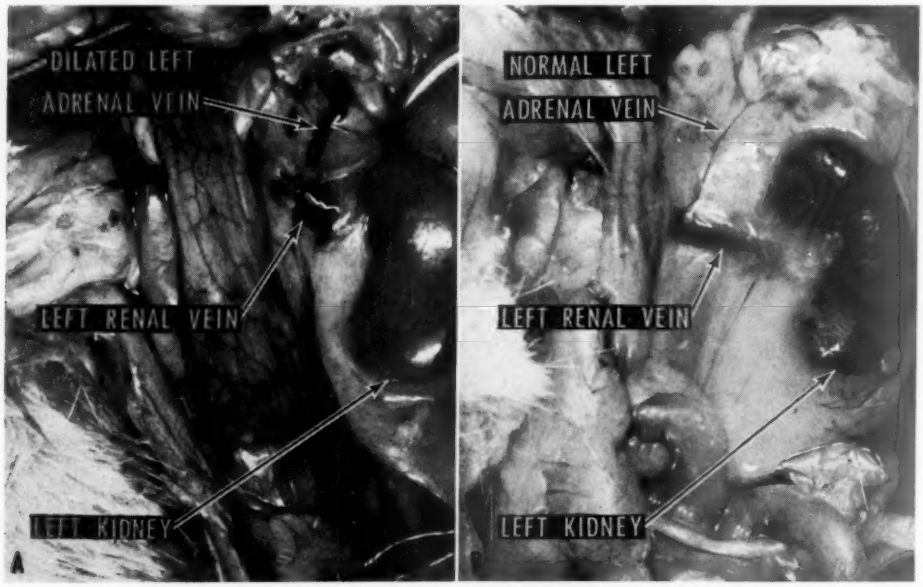

Fig. 11.—These are 2 photographs showing the adrenal-renal communication established between the portal and systemic circulations. *A* shows these vessels in a cirrhotic rat with portal hypertension. *B* depicts their normal appearance in a control animal.

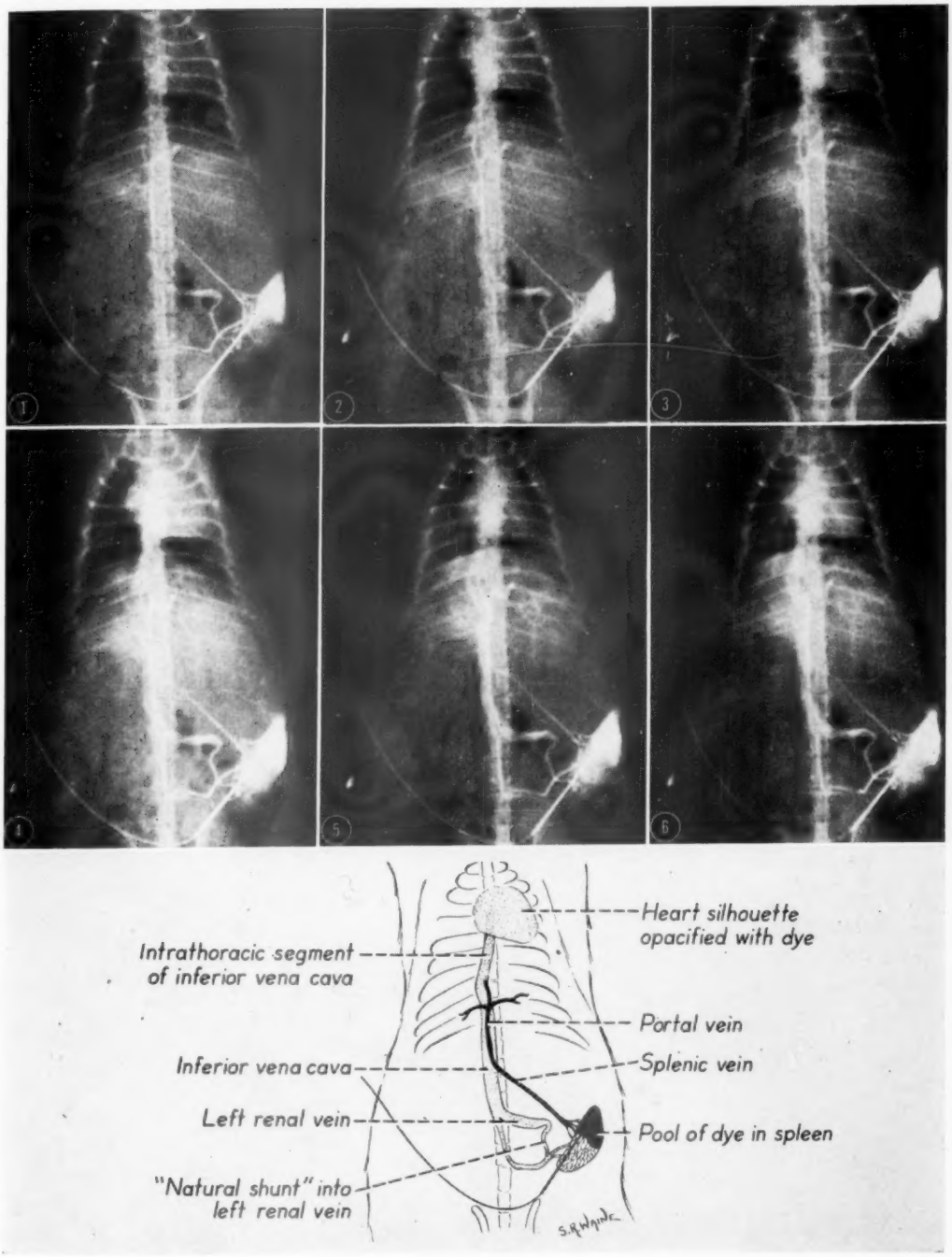

Fig. 12.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Dye from the splenic pool shows limited filling of the splenoportal axis and intrahepatic vasculature. Most of the dye, however, is diverted from the liver via perihilar collaterals which take portal blood into the inferior vena cava via the left renal vein and via an iliolumbar vein. Since most of the dye is diverted through "natural shunts," very little filling of sinusoidal capillaries can be observed in the hepatogram phase.

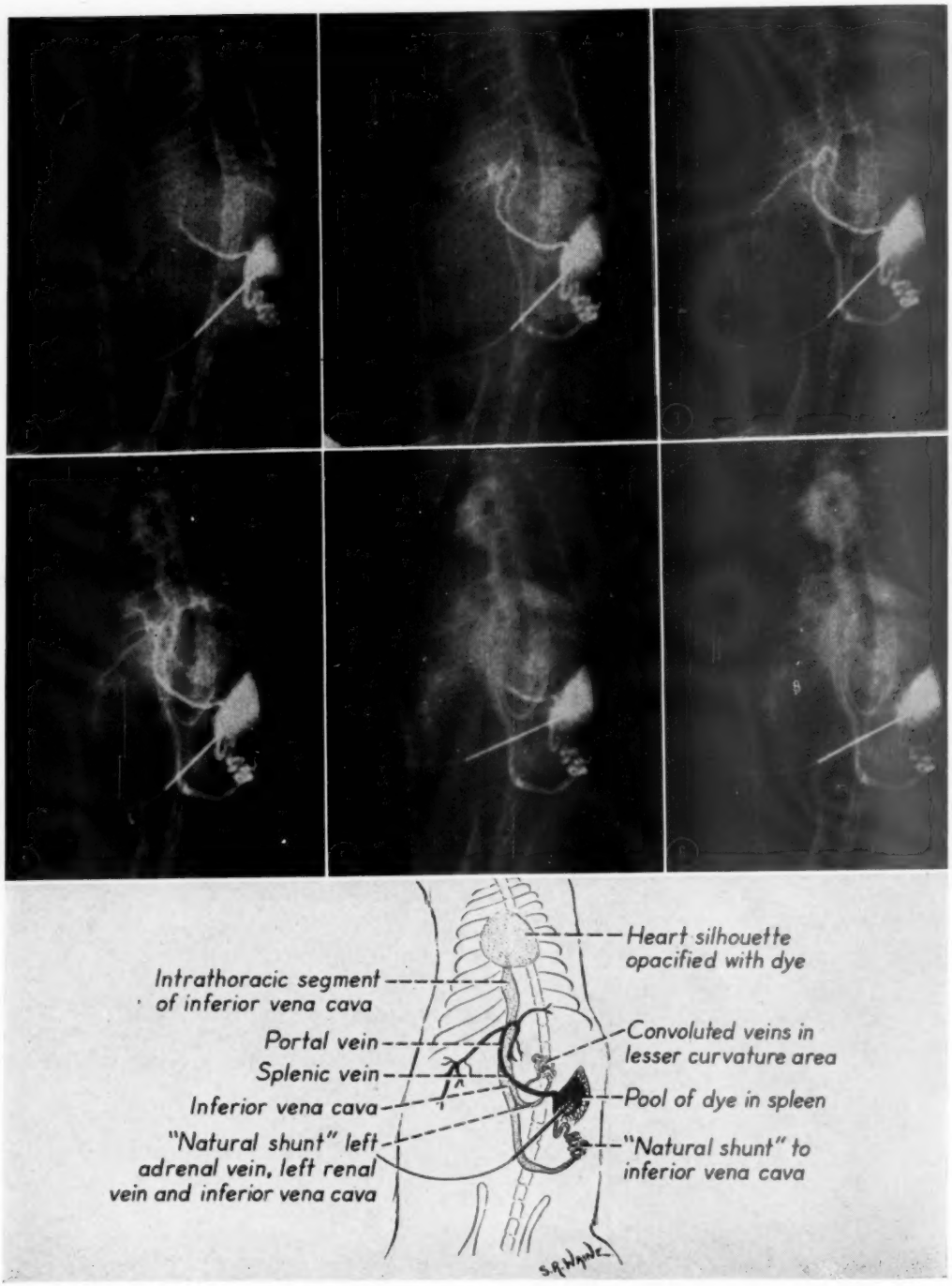
Heart silhouette opacified with dye
Intrathoracic segment of inferior vena cava
Portal vein
Splenic vein
Inferior vena cava
"Natural shunt" left adrenal vein, left renal vein and inferior vena cava
Convoluted veins in lesser curvature area
Pool of dye in spleen
"Natural shunt" to inferior vena cava

Fig. 13.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Dye from the splenic pool fills the splenoportal axis and the intrahepatic vasculature. There is a small network of varicosities in the area of the lesser curvature of the stomach. From this last area, the left adrenal and left renal veins are filled, the latter emptying into the inferior vena cava. From the hilum of the spleen a very tortuous vessel courses downward to fill the iliolumbar vein which in turn empties into the inferior vena cava. The confluence between the inferior vena cava and the left renal vein is thus clearly demonstrated as is the phenomenon of streamlining at the point where the 2 streams of blood meet. Progressive opacification of the cardiac silhouette is evident. An irregular hepatogram phase is observed.

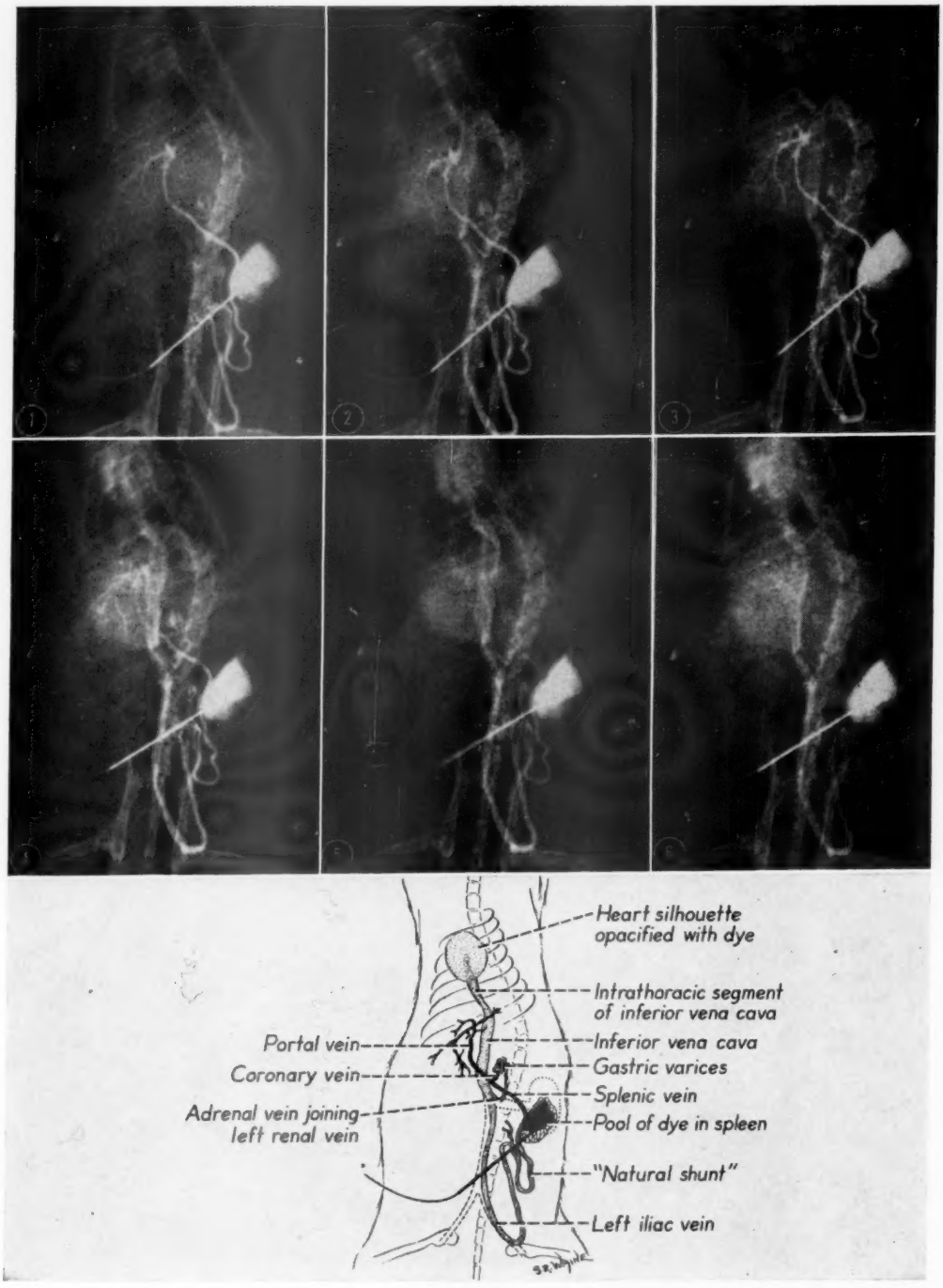

Fig. 14.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Splenic vein, portal vein, and intrahepatic branches are visualized. Coronary vein arises from the portal vein and fills a small network of convoluted veins in the area of the lesser curvature of the stomach. Left adrenal vein receives portal blood from retrogastric plexuses and empties it into the inferior vena cava via the left renal vein. From the hilum of the spleen a collateral vein follows a downward, tortuous course to end in the left iliac vein which in turn fills the inferior vena cava.

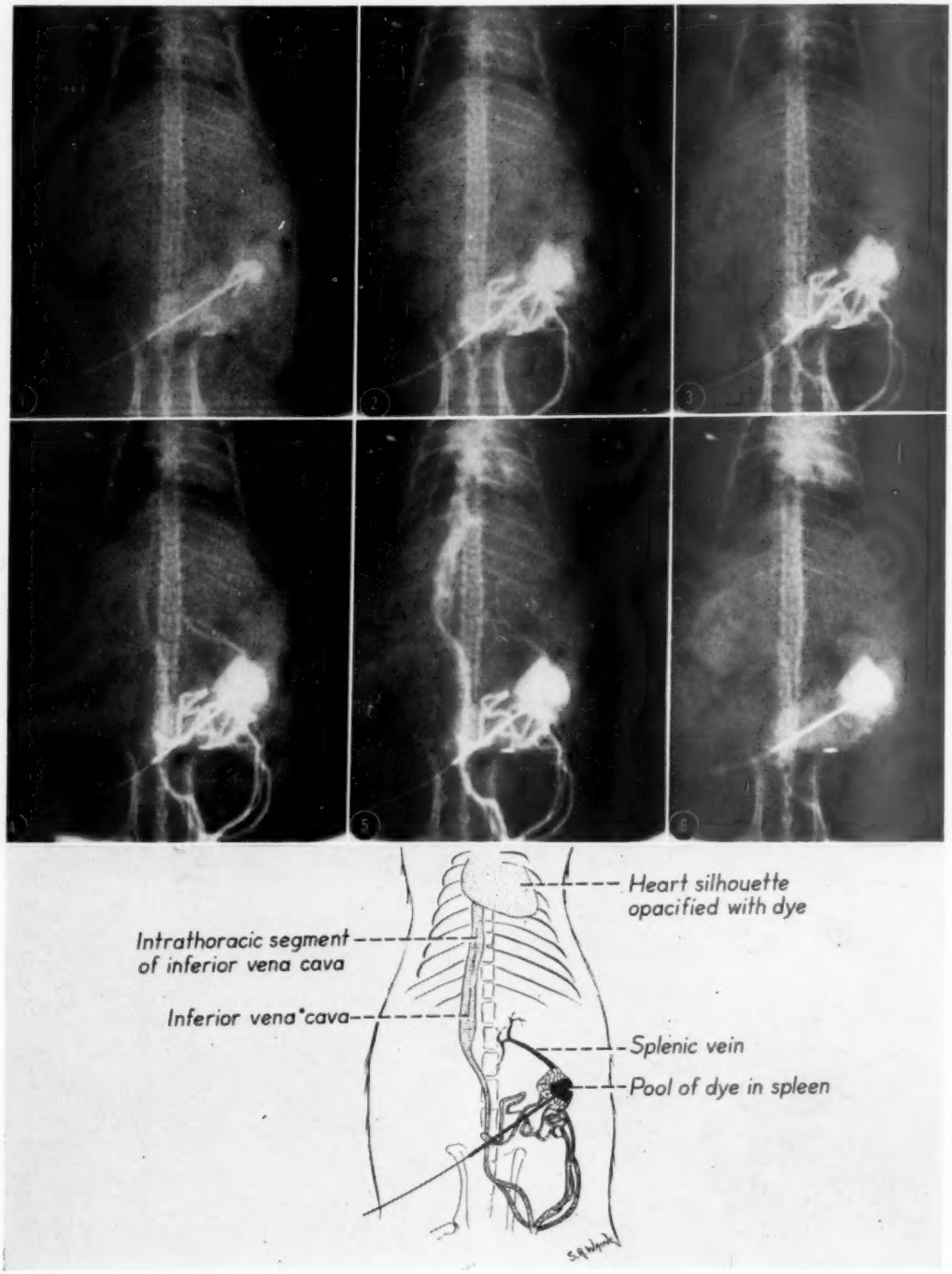
Heart silhouette opacified with dye
Intrathoracic segment of inferior vena cava
Inferior vena cava
Splenic vein
Pool of dye in spleen

Fig. 15.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. Dye deposited in the splenic pulp is diverted in a downward direction by a system of convoluted veins arising in the hilum of the spleen and emptying into the left iliac vein and the inferior vena cava. This last vessel is visualized in its entire length in film 5. The splenoportal axis is not filled except for a portion of the splenic vein. No dye reaches the liver as is shown by the lack of visualization of the intrahepatic vasculature and the complete absence of a hepatogram phase. A certain degree of improvement in this hemodynamic situation followed the interruption of the administration of the toxic agent for a period of 90 days as can be observed in Fig. 21.

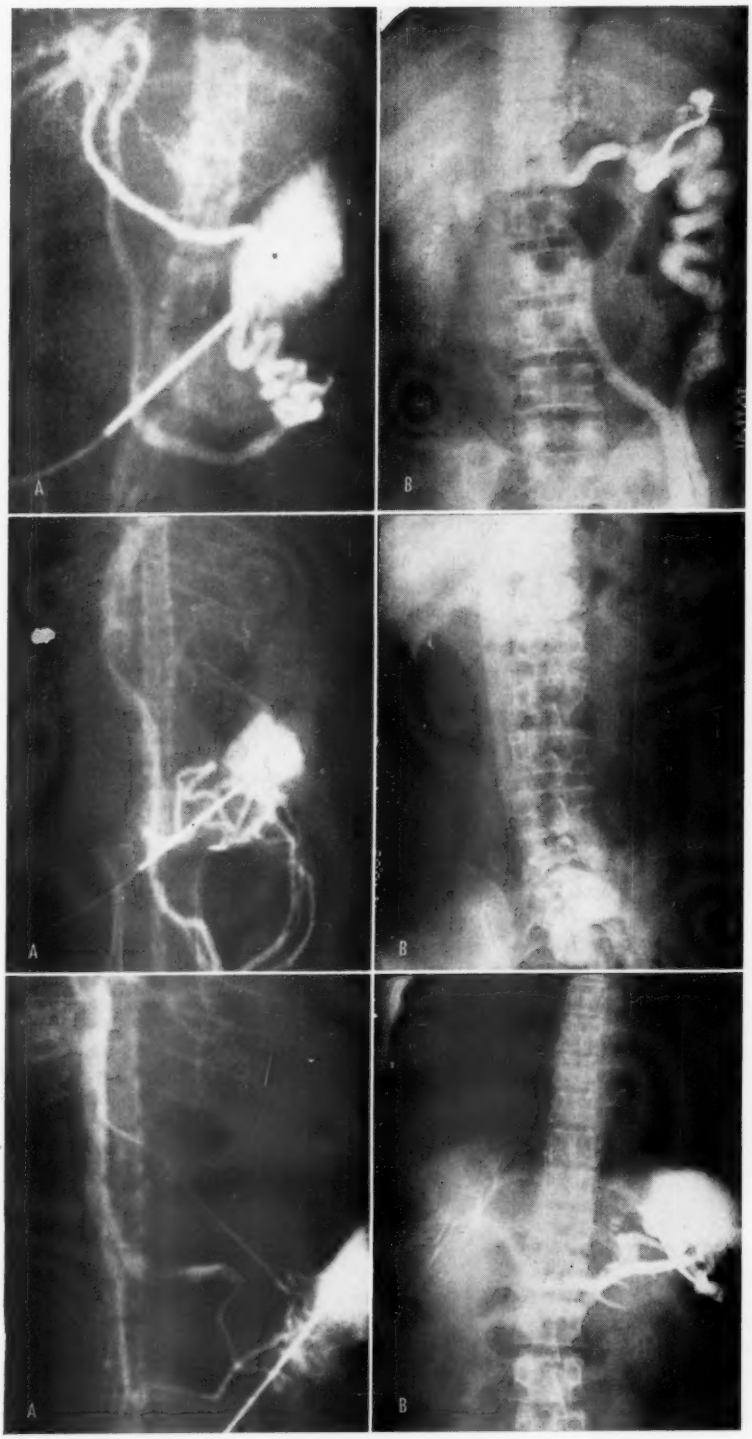

Fig. 16.—A comparison between the radiologic appearance of a natural shunt mediated through a convoluted splenic hilar branch and the vena cava. *A*, Film from a splenoportogram of a cirrhotic rat. *B*, Film from a splenoportogram of a human patient with portal hypertension.

Fig. 17.—A comparison between the radiologic appearance of a natural shunt communicating with the left iliac vein. *A*, Film from a splenoportogram of a cirrhotic rat. *B*, Film from a splenoportogram of a human patient with portal hypertension showing a similar but not identical type of communication.

Fig. 18.—A comparision between the radiologic appearance of the communication of the perihilar splenic branch with the left renal vein. *A*, Film from a splenoportogram of a cirrhotic rat. *B*, Film from a splenoportogram of a human patient with portal hypertension.

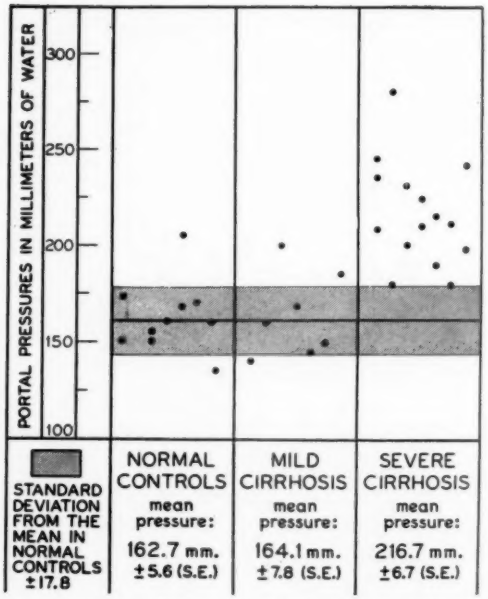

Fig. 19.—A graph showing the values of portal pressures in rats in the normal control group, in the group of animals with mild cirrhosis, and in the group with severe cirrhosis. The difference between the mean value of portal pressure of the control group and that of the group with severe cirrhosis was statistically highly significant, $P < 0.001$.

TABLE II. PORTAL PRESSURE IN NORMAL AND CIRRHOTIC RATS

GROUP	ANIMAL NUMBER	PORTAL PRESSURE	MEAN PRESSURE	STANDARD ERROR	STANDARD DEVIATION
Normal controls	32N1	150			
	58N1	155			
	58N2	160			
	8N4	205	162.7	±5.6	±17.8
	56N1	170			
	56N2	160			
	56N3	174			
	56N4	150			
		168			
		135			
Mild cirrhosis	14B2	160			
	15B1	200			
	15B2	168			
	15B3	145	164.1	±7.8	±20.5
	16B1	150			
	19B1	186			
	29C4	140			
Severe cirrhosis	20C2	235			
	21C2	280			
	22C3	232			
	23C2	210			
	25C1	190			
	25C3	180			
	26C3	242	216.7	±6.7	±26.1
	27B1	245			
	28C3	180			
	29C1	200			
	30C1	215			
	30C3	212			
	51D4	198			
	51D1	208			
	51D2	224			

The difference between the mean pressure in the normal control group and the mean pressure in the group of animals with severe cirrhosis is statistically highly significant, $P < 0.001$.

altered hemodynamics was observed in only 17 per cent of the cases (Figs. 20 and 21). However, no improvement or even further progression of the circulatory derangement was observed in the majority of the remaining cases.

DISCUSSION

A complete state of portal hypertension similar to that observed in man has developed in a large colony of rats with experimentally-induced cirrhosis of the liver. The similarity in the basic mechanisms of the hemodynamic response has manifested itself both by a sustained elevation in portal pressure and by an active hepatic bypass of portal blood. The pathways of portal

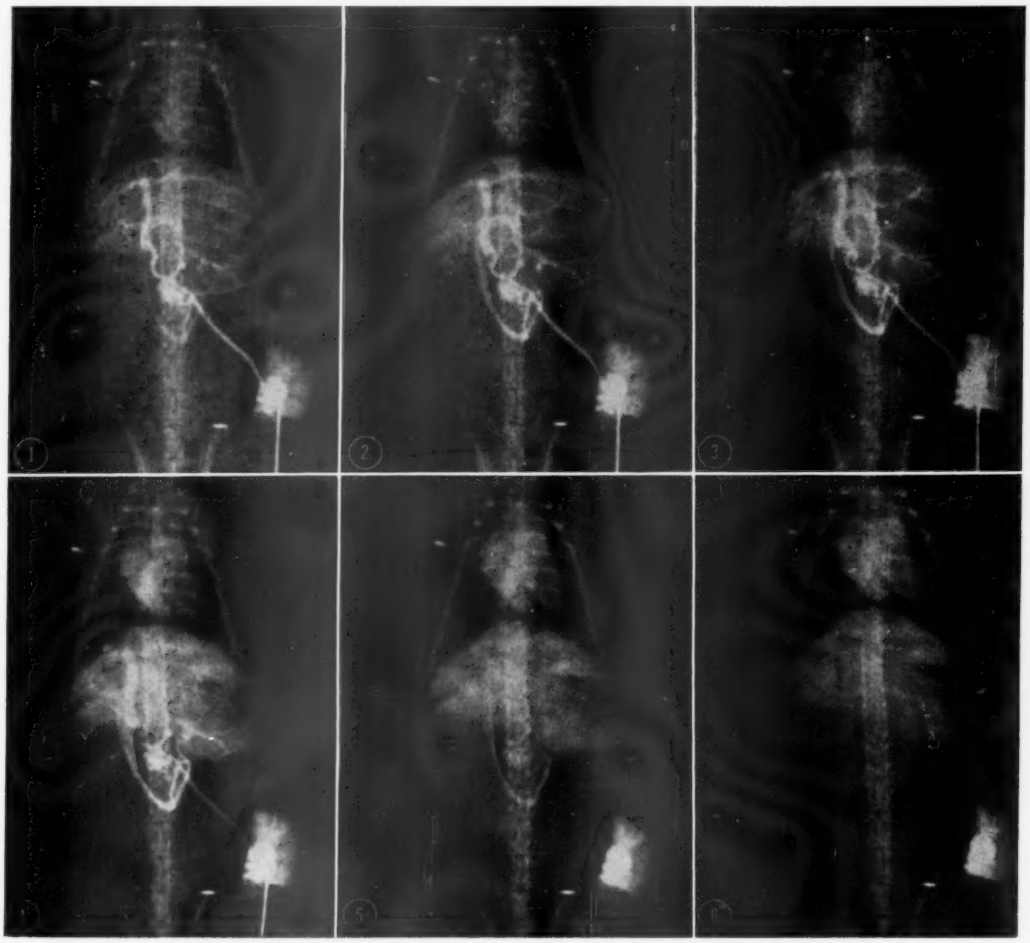

Fig. 20.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. This splenoportogram corresponds to the same animal depicted in Fig. 5. It was taken 90 days after the interruption of the hepatotoxic agent and shows improvement in the hemodynamic conditions observed in the previous one, although abnormal features still persist. In this portogram the splenic vein fills a distorted portal vein and its intrahepatic branches. Dilation of these vessels is less marked than in the previous splenoportogram. Filling the coronary vein and gastric plexuses persists but esophageal varicosities have completely disappeared. The natural shunt mediated by the left adrenal and renal veins also persists but a considerably lesser amount of dye is diverted through these pathways. This can better be estimated by comparing the degree of filling of the inferior vena cava in the previous splenoportogram. The left renal vein is actually not visualized in this case beyond the point of confluence with the adrenal vein. The increased amount of portal blood reaching the liver at this time is demonstrated by a markedly denser hepatogram phase (films 4-6). Increased opacification of the heart is observed.

diversion have not differed from those observed in human patients with portal hypertension.

Evidence presented in this paper appears to contradict the generalized opinion that the syndrome of portal hypertension, as seen in man, is not reproducible in the experimental animal. Whether the hemodynamic changes observed in this investigation were related to the method of inducing cirrhosis, i.e., direct introduction of the hepatotoxic agent in the gastrointestinal tract,

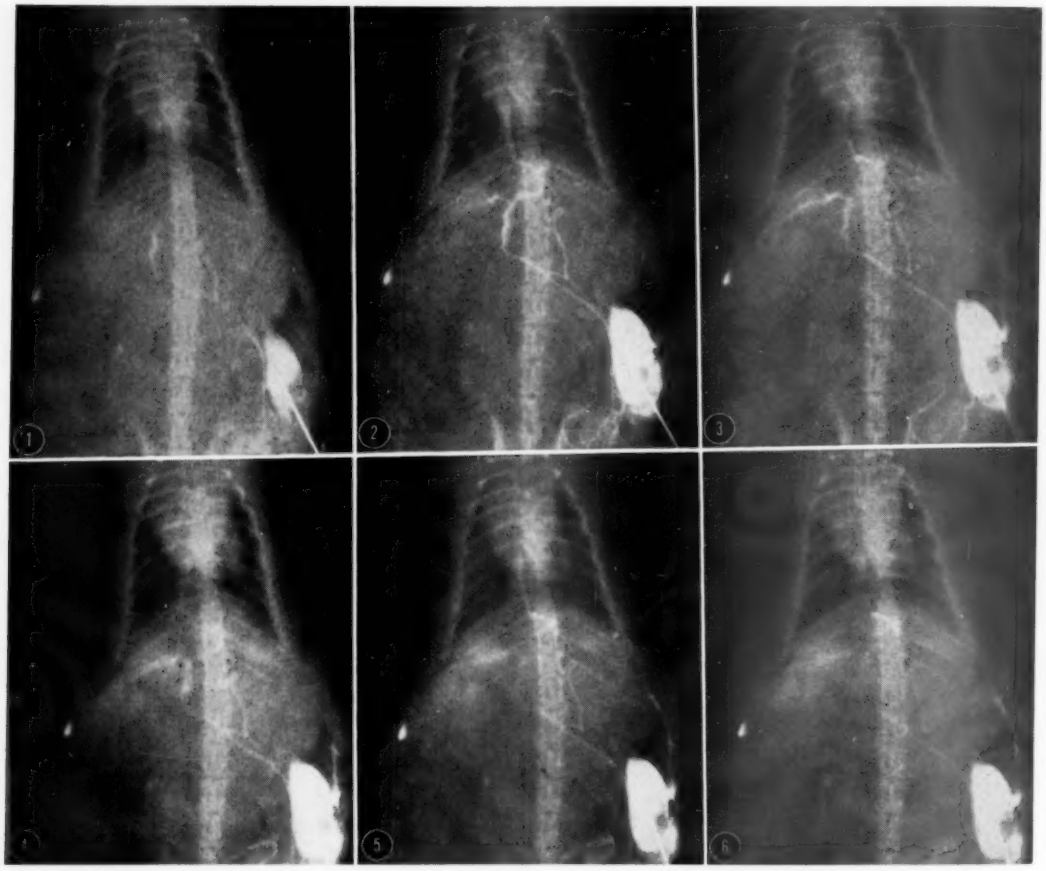

Fig. 21.—Six films selected from a 15-film, multiple-exposure splenoportogram in a cirrhotic rat. This splenoportogram corresponds to the same animal depicted in Fig. 15. It was taken 90 days after the interruption of the hepatotoxic agent and shows improvement in the hemodynamic conditions observed in the previous films, although abnormal features still persist. The dye deposited in the spleen now reaches the liver via the splenoportal axis in approximately normal fashion. Although circulation through downward perihilar collaterals still persists it does not produce visualization of the inferior vena cava as was the case in the previous splenoportogram. Intrahepatic vasculature is now filled and an irregular hepatogram phase can be discerned particularly on films 5 and 6 (in contrast to complete absence in previous portogram). A coronary vein is visualized and delivers portal blood to the right heart via the azygos system. Opacification of the heart is not as sharply defined as in the previous splenoportogram.

or to the more complete in vivo evaluation of the portal system provided by the extensive use of splenoportography not reported before, cannot be established at this time.

It was of interest to observe that a small percentage of cirrhotic animals did not show abnormalities in the splenoportograms, a situation which was also observed in cirrhotic patients.[17]

The incidence of distortion of the intrahepatic branches of the portal vein, as well as the frequency of the abnormal hepatogram phase, closely paralleled those observed in human beings.[17]

Hepatic bypass of portal blood was effected through the 2 main routes observed in man: (1) by reversed flow into branches of the portal system, and (2) through larger portal systemic communications (natural shunts).[14] In the first type of bypass, the coronary vein, its gastric tributaries, the esophageal veins, and the superior mesenteric vein were the main pathways of diversion. The predominance of varicosities in the region of the cardia of the stomach over those observed in the esophagus compares favorably with the corresponding incidence in human splenoportograms.[15, 18] In the second type, natural shunts delivering portal blood into the left renal vein, into the iliolumbar veins or into the iliac vein were no different from those observed in man.[14]

One type of natural shunt connecting the perigastric plexuses in the region of the cardia of the stomach with the left adrenal and renal veins was so frequently observed that the conclusion that this communication normally exists in the majority of the animals seemed unavoidable. An identical connecting channel has also been described in the cadaver of a patient with a normal liver.[7] It is possible that when portal hypertension develops, both in man and in the experimental animal, this pre-existing portasystemic communication is activated and dilated by retrograde flow of portal blood. It is also the opinion of the present authors, based upon observations of splenoportograms, that the adrenal vein is one of the vessels which communicates with the left renal vein in many patients with portal hypertension.

This phenomenon of hepatic bypass of the portal blood, effected through a variety of pathways, is difficult to explain on a basis other than the presence of an intrahepatic block to the portal circulation. The nature of the obstructing mechanism may be either anatomic (compression of small intrahepatic branches of the portal and hepatic veins), functional (intrahepatic arteriovenous fistulas), or a combination of both. At any rate, the portasystemic collateral pathways over which this bypass of the liver occurs resemble in every respect the well-described bridging collaterals observed in association with circulatory obstruction seen elsewhere in the peripheral arterial and venous systems.

Again it should be noted that large natural portasystemic venous shunts have neither prevented the occurrence of gastroesophageal varices nor decompressed the portal system to a degree that varices cannot be demonstrated. This failure of adequate decompression is felt to be due both to the tortuosity of these vessels and also to the low flow rates through them.[14]

The sustained elevation in portal pressure required as an essential component of the state of portal hypertension has been obtained in this experiment, and has been also reported by other investigators.[2, 8, 20] The readings of portal pressure obtained in the control group in this investigation appeared to be somewhat higher than those previously reported as normal[3] and this may

be caused by the use of a larger (No. 18 gauge) needle which perhaps prevented interference from the occurrence of capillary phenomena known to take place in small-bore tubes.

It is of further interest that esophagogastric bleeding was not observed in any of the animals with varices. This dissimilarity with the human syndrome of portal hypertension may perhaps be explained on an anatomic basis. In the rat, dilated gastroesophageal veins are observed only over the serosal surfaces and in tissues surrounding the alimentary tract, whereas in human beings these veins are commonly prominent in the esophageal and gastric submucosal layer. This latter location, perhaps, makes these thin-walled varicosities vulnerable because of the relatively inadequate protection afforded by the thin mucosal layer.

Although ascites was present in 59 per cent of the colony of cirrhotic rats, no further elucidation concerning the mechanisms of formation of ascitic fluid was derived from this study.

It was noted that in the majority of animals there was no amelioration of the portal circulatory alterations after discontinuance of the hepatotoxic agent. In a small percentage, however, definite improvement was noted. If, in the future, a better understanding of the etiological factors responsible for human cirrhosis is realized, the associated portal hypertension in at least some of these patients may be reversed by methods other than surgical.

We are deeply indebted to Dr. Francis F. Ruzicka, Jr., Director of Radiology, to Dr. Antonio Rottino, Director of Pathology, to Dr. Raymond Gettinger of the Pathology Department, to Dr. Arthur Levy, Director of the Biochemistry Laboratory, St. Vincent's Hospital, New York City.

REFERENCES

1. Abeatici, S., and Campi, L.: Sur les possibilites de l'angiographie hepatique. La visualization du systeme portal, Acta radiol. **36**: 383, 1951.
2. Daniel, P. M., Prichard, M. M. L., and Reynell, P. C.: The Portal Circulation in Experimental Cirrhosis of the Liver, J. Path. & Bact. **64**: 53, 1952.
3. Farris, E. J., and Griffith, J. Q., Jr., editors: The Rat in Laboratory Investigation, Philadelphia, 1949, J. B. Lippincott Company, p. 288.
4. Gershbein, L. L., and Elias, H.: Observations on the Anatomy of the Rat Liver, Anat. Rec. **120**: 85, 1954.
5. Islami, A. H., and Pack, G. T.: Demonstration of Liver Regeneration by Splenoportal Venography, Am. J. Roentgenol. **81**: 855, 1959.
6. Liebowitz, H. R., and Rousselot, L. M.: Bleeding Esophageal Varices. Portal Hypertension, Springfield, Ill., 1959, Charles C Thomas, Publisher, pp. 276, 286, 287.
7. Madden, J. L., Loré, J. M., Gerold, F. P., Ravid, J. M.: The Pathogenesis of Ascites and a Consideration of Its Treatment, Surg. Gynec. & Obst. **99**: 385, 1954.
8. Mangalik, V. S., Mehrotra, R. M. L., and Nayak, N. C.: The Pathogenesis of Ascites in Carbon Tetrachloride Cirrhosis, J. Path. & Bact. **73**: 239, 1957.
9. Moreno, A. H., Chiesa, D., and Affani, J.: Studies on the Portal Tension of Human Adults, Surg. Gynec. & Obst. **104**: 25, 1957.
10. Moreno, A. H., Rousselot, L. M., and Panke, W. F.: Studies on Portal Hypertension. II. Correlation Between Severity of Pathologic Involvement of the Portal System and Variations in Tension, S. Clin. North America **38**: 421, 1958.
11. Moreno, A. H., Rousselot, L. M., and Panke, W. F.: Studies on Portal Hypertension. III. The Rate of Hepatic Blood Flow in Normal Subjects and in Patients with Portal Hypertension. In press.
12. Natelson, S.: Microtechniques of Clinical Chemistry for the Routine Laboratory, Springfield, 1957, Charles C Thomas, Publisher, pp. 132-134.
13. Popper, H., and Schaffner, F.: Liver: Structure and Function, New York, 1957, McGraw-Hill Book Company, Inc., p. 286.

14. Rousselot, L. M., Moreno, A. H., and Panke, W. F.: Studies on Portal Hypertension. IV. The Clinical and Physiopathologic Significance of Self-Established (Nonsurgical) Portal Systemic Venous Shunts, Ann. Surg. **150**: 384, 1959.
15. Rousselot, L. M., Ruzicka, F. F., Jr., and Doehner, G. A.: Portography in Portal Hypertension. Its Application in Diagnosis and Surgical Planning, S. Clin. North America **36**: 361, 1956.
16. Rousselot, L. M., and Thompson, W. P.: Experimental Production of Congestive Splenomegaly, Proc. Soc. Exper. Biol. & Med. **40**: 705, 1939.
17. Ruzicka, F. F., Jr., Bradley, E. G., and Rousselot, L. M.: Two-Phase Opacification of the Liver in Cirrhosis, Ann. New York Acad. Sc. **78**: 819, 1959.
18. Ruzicka, F. F., Jr., Doehner, G. A., and Rousselot, L. M.: Portal Venography. Anatomic and Physiologic Considerations in Interpretation, Am. J. Digest. Dis. **1**: 3, 1956.
19. Taylor, F. W.: Experimental Portal Hypertension, Ann. Surg. **146**: 683, 1957.
20. Warren, K., and DeWitt, W. B.: Production of Portal Hypertension and Esophageal Varices in the Mouse, Proc. Soc. Exper. Biol. & Med. **98**: 99, 1958.
21. Wiles, C. E., Jr., Schenk, W. G., Jr., and Lindenberg, J.: The Experimental Production of Portal Hypertension, Ann. Surg. **136**: 811, 1952.

(For discussion, see page 152.)

THE PORTACAVAL SHUNT OPERATION IN PATIENTS WITH CIRRHOSIS AND ASCITES

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DESPITE wide interest and investigation concerning the relationship between portal hypertension and ascites formation, the relationship remains incompletely understood. It is now usually accepted that the circulatory abnormality in cirrhosis is a partial obstruction to outflow of blood from the liver caused by intrahepatic compression of tributaries of the hepatic veins by regenerating liver nodules.[14, 18] This leads to congestion of the liver and to a rise in portal pressure both intra- and prehepatically. Some authors have suggested that the hypertension represents transmission of arterial tension (hepatic artery) to the portal venous system both intra- and extrahepatically either with or without the operation of arteriovenous shunts. Others mention only the obstruction to flow and the congestion, and believe that pressure would build up behind an obstruction in any case. While transmission of arterial pressure may be operative under some circumstances it is not necessary in all cases as illustrated by the high pressures developed by patients with extrahepatic block. The traditional view has held that, in spite of the intrahepatic obstruction, at least some of the blood in the portal vein continues to circulate through the liver. This has been thought to be beneficial and for years Johnston and colleagues[16] have advocated the side-to-side shunt to preserve this cephalad flow. Likewise, and for the same reason, Blakemore[2] has advocated the side-to-side shunt in selected patients when there was evidence from pressure measurements at operation that a considerable portal flow was still going through the liver.† When such evidence has been lacking he has preferred the end-to-side shunt. Information is now accumulating suggesting that among the latter group there are some patients in whom the forward flow through the portal vein has not only ceased but has actually become retrograde so that it affords an accessory outflow tract from the liver.[4, 12, 17, 29, 31] After the construction of a side-to-side shunt it is likely that the direction of flow is usually, if not always, retrograde even in patients who had a forward (cephalad) flow before the shunt was created.[29]

The observation that patients with extrahepatic block rarely develop ascites (although a few instances have been reported[1]), has frequently been

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†Compression of the intact portal vein led to further rise of at least 150 mm. H_2O in portal pressure caudad to the compression.

cited to support one view or another. Authors commonly use it as evidence for the belief that the site of production of ascitic fluid is from the surface of the liver rather than from the more peripheral splanchnic surfaces. Other authors are of the opinion that the normal hepatic function in patients with extrahepatic block, the better serum protein levels, and perhaps better conjugation of adrenal cortical steroids, may explain at least in part the lack of ascites. That the latter facts may be irrelevant is suggested by the massive ascites which patients with Budd-Chiari syndrome develop in spite of a normal (but congested) liver. In any event there is other evidence accumulating that the major source of ascitic fluid production is from the surface of the liver, i.e., from the area of intrahepatic portal hypertension.[8, 13, 19] The correction of ascites would therefore seem to be accomplished most directly by reduction of the intrahepatic pressure. The use of the side-to-side shunt (affording a second outflow tract) has thus logically been proposed for treatment of ascites.[20, 29, 30, 31] Certainly, its use should produce the maximum attainable drop in intrahepatic pressure. An end-to-side shunt for treatment of ascites,[6, 7, 10, 25] while probably reducing the volume flow of blood through the liver in those patients who formerly had a cephalad flow through the portal vein could, however, actually increase the congestion by removing the accessory outflow route in patients who had had retrograde portal flow preoperatively (assuming the arterial flow remained unchanged). This destruction of an outflow tract should (in the latter group) increase the intrahepatic portal pressure and the tendency for ascites to develop. A considerable percentage of patients with cirrhosis who have advanced ascites are thought to exhibit such retrograde flow[12, 29] before shunts are made. By constructing shunts one might expect to make the ascites worse, or even produce ascites in some patients who never had ascites before. Thus, on theoretical grounds, one is tempted to condemn the end-to-side shunt. Based on these considerations, several authors have recently reported their experiences and advocated the use of side-to-side shunts for the treatment of ascites.[20, 29-31]

It is interesting to recall that end-to-side shunts have been created in hundreds of patients without any reported tendency to produce ascites. In fact, protection seems to be afforded against ascites development in some patients in whom the cirrhosis continues to advance with increasing outflow block. Furthermore, a great many of these patients have had mild ascites preoperatively which never recurred following the operation. This observation is sometimes dismissed on the basis that the ascites usually encountered is mild and will respond to diuretics or simple salt restriction, or that following the operation the patients ate better and their serum protein levels might have improved. It is commonly held that while hypoproteinemia alone is not likely to produce ascites, it is a contributing factor along with portal hypertension. Actually, it is unusual for postoperative patients with cirrhosis to have improvement of their serum protein levels for many months.

Our interest at Presbyterian Hospital in this subject began about 8 years ago when our early end-to-side shunts for severe ascites were performed.

TABLE I. CIRRHOSIS WITH MEDICALLY IRREVERSIBLE ASCITES

PATIENT	AGE	SEX	HISTORY		DURATION OF ASCITES (MO.)	DATE OF SHUNT	TYPE	PORTAL PRESSURES (MM. H_2O)			
			ALCOHOL	HEPATITIS				OPEN INTACT PORTAL VEIN	OBSTRUCTED PORTAL VEIN	SHUNT OPEN	SHUNT OBSTRUCTED
1. C. M.	52	M			12	1/15/52	E-S	350	420	310	500
2. H. H. L.	52	F	X		3	5/27/52	S-R	390		270	270
3. L. B.	48	M	X		36	1/13/53	E-S	345	445	230	
4. E. S.	47	F	X		14	7/15/53	E-S	410	450	340	600
5. H. L. L.	35	F	X	X	7	1/ 5/54	E-S			236	450
6. M. M.	49	F	X		24	2/ 9/54	E-S				
7. P. DeS.	37	M			14	3/ 2/54	S-S	350	500		
8. J. E.	48	M	X		10	3/ 1/55	E-S				
9. A. B.	56	F			20	3/31/55	E-S	290	290	180	
10. H. C.	50	F			3	11/23/55	E-S			150	510
11. M. G.	44	F	X		10	3/28/56	E-S				
12. J. S.	64	M	X		19	12/19/56	E-S	260	340	190	320
13. M. H.	59	F			5	7/17/57	E-S	400		170	480
14. C. C.	41	M	X	X	5	3/19/58	E-S	340	340	220	380
15. A. W.	34	M	X		18	6/ 3/59	E-S	410	410	260	
Median	48			Median	12						

Following the publication[10] in 1953 of some of these early experiences, it was decided to conduct a definitive study to determine whether or not shunting was useful in the treatment of ascites. At that time the question of which type of shunt was best had not yet developed. It was well known that prolonged, intelligent, nonsurgical treatment would result in the resolution of ascites in a considerable number of patients. It therefore seemed to us from the outset that there were only 2 ways to determine the usefulness in patients of shunting in treatment of ascites. The first would be to collect a very large experience of matched (or alternate) cases treated with and without operation and compare the results. If more or less mild cases of ascites were to be included it seemed that attainment of statistical significance might require at least several dozen cases, carefully matched. The other approach seemed to be to work with a highly selected group of patients in whom it could be predicted in advance that the majority would not respond to nonsurgical treatment. These would have to represent the most severely medically irreversible* cases of ascites we could find and it would be inevitable that most of them would be high surgical risk patients from the standpoint of over-all liver function. We would thus be accepting a high operative mortality rate and early postoperative deaths from liver failure. The selected series approach seemed to offer the best chance of settling the question of the usefulness of shunting in the treatment of ascites.

MATERIAL AND METHODS

In an effort to select patients for study with only the most medically irreversible ascites, we established rigid criteria for admission to the series. Three patients from the previously published 15[10] seemed satisfactory and 12 have been added since (Table I). All patients included had had massive ascites with a minimum duration of 3 months. The median duration for the series was 12 months. A history of preadmission paracenteses was required but one of hemorrhage or demonstrable varices was not. The most exacting criterion of irreversibility seemed to be a very low urinary sodium excretion. Accepted patients all showed little or no response of urinary sodium excretion to mercurial diuretics and their 24-hour output was under 5 mEq. even when unlimited sodium intake was permitted. With a very low sodium diet the urinary output was usually under 1 mEq. per 24 hours. Brief periods of higher outputs were recorded in several patients on a variety of drugs but if these occurred following administration of mercurials, the patient was not accepted for the study series (Table II).

Balance studies were kept of all patients who met the above criteria throughout their hospital course. Those who were eventually operated upon had all been studied for a minimum of 4 weeks preoperatively and in some instances for several months. A considerable number of other patients were studied in this manner for extended periods, but never operated upon because of excessive surgical risk, usually based on liver function tests and clinical assessment. It is our belief that a good appetite and clinical appearance are sometimes more important than chemical tests of liver function. The cessation of ascites formation on institution of a low salt diet is a favorable sign. Several seemingly high risk patients were operated upon when there were unusually strong operative indications (history of multiple, recent, massive hemorrhages). The method of selection has obviously weighted the series with moderately poor risk patients by eliminating those who were the best risks and most of those who were the very worst risks.

In retrospect, it appears that prolonged preoperative preparation could not be counted upon to improve appreciably the chemical tests of liver function. Spontaneous improvement

*We prefer this term to the usual "intractable" which has been used so often without documentation that it no longer has meaning when applied to ascites.

in albumin levels and prothrombin time did not ordinarily occur, but both of these were specifically corrected as far as possible just prior to operation. When jaundice was present, however, considerable fluctuation was found to occur in the bilirubin levels and we have felt that it is best to wait for jaundice to subside before operation. Since ascitic patients all possess large excesses of total body sodium we have felt that they would benefit by reduction of these stores preoperatively. In addition to the low salt intake (generally about 10 mEq. of sodium per day) we have therefore used occasional paracenteses and/or a variety of diuretic preparations. Resins have not been employed in recent years although they were used in a few of the early patients. Many of the patients showed a slight to moderate hyponatremia on admission and it often gradually deepened with sodium withdrawal. Simultaneously, the formation of additional ascites generally stopped and weight stabilized. The actual reabsorption of ascites was rarely encountered in the present series even with careful in-hospital management. The practice of sodium withdrawal preoperatively is based on an assumption that it will be beneficial but we have no objective evidence on this point.

The median age of the patients was 48 years with a range of 34 to 64. Seven were men; 8 women. All had cirrhosis and in 10 there was a good history of excessive alcohol intake. In 2 there was a history of hepatitis. In 5 there was no history of either alcohol or hepatitis. In 1 patient a side-to-side shunt was performed because of our belief at

TABLE II. PREOPERATIVE URINARY SODIUM EXCRETION (MEQ./24 HOURS)

PATIENT	PREOPERATIVE WEEKS								
	9	8	7	6	5	4	3	2	1
1.	4.4	0	2.7	1.9	0	0	2.0	0	7.8
2.						0	0	0	0
3.				0	0	0	0	0	0
4.						0	0	0	0
5.						0	1.6	13.1	4.8
6.						0	1.9	0	8.1
7.						0	0	0	0
8.					0	0	0	0	0
9.		0	0	0	0	0	0	0	0
10.	2.0	1.5	3.2	9.1	1.0	42.2	13.0	13.8	13.5
11.	4.6	5.6	6.3	1.3	2.3	1.0	1.8	26.1	11.5
12.	0	0	0	0	0	1.4	0	0	0
13.						0	0	0	0
14.					1.1	0	0	0	0
15.	1.0	1.3	2.3	2.4	2.6	4.9	1.5	2.1	2.8

Note: 0 designates an excretion of less than 1 mEq. per 24 hours. Intake throughout was approximately 10 mEq. sodium per 24 hours.

that time that a marked increase in portal pressure on compression of the vein suggested the inadvisability of a complete diversion of portal blood away from the liver. In another patient a splenorenal shunt was made because of the presence of an extremely large caudate lobe of the liver. The other 13 patients all underwent end-to-side shunts of portal vein to vena cava.

The operation was usually performed through a right thoracoabdominal incision. In a few instances hypotensive anesthesia was used. Hypothermia was not used in the present series. Postoperatively the patients were kept on a low sodium intake (10 mEq. per 24 hours) until they started to excrete 10 mEq. or more daily in the urine. This practice was again based on the assumption that it would be beneficial to eventually reduce total body sodium to normal. It should be mentioned that on this program the patients did not develop apparent ascites in the postoperative period. Most of the patients remained somewhat hyponatremic (without apparent ill effect) during the early postoperative period. In a few instances the serum sodium levels were observed to fall progressively to a point requiring the administration of sodium. Serum sodium levels as low as 122 mEq. per liter, however, were generally well tolerated and were not taken per se as an indication for liberalizing the sodium intake.

Following the onset of sodium diuresis, the sodium intake was progressively raised so that some patients were discharged on diets of 40 mEq. per day or more. In others a quick clinical recovery made it impractical to keep them in the hospital to wait for sodium diuresis and they were sent home on a diet of 10 to 20 mEq. daily. In all such instances the patients were readmitted for study at a later date and ability to excrete sodium in adequate amounts was demonstrated by balance techniques (Table III).

Total 24-hour urinary sodium output was determined throughout the hospital course for all patients. For compilation of the tables the data were combined into weekly periods and the median day for each weekly period was selected as "typical" for that week. This method is believed to be superior to taking the mean since the distribution this near to zero output is highly skewed.

TABLE III. POSTOPERATIVE URINARY SODIUM EXCRETION (MEQ./24 HOURS)

PATIENT	WEEKS POSTOPERATIVE														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3.	0	0				24.3			23.9	26.3	41.2				
4.	0	0	Readmitted 3 years later; excreting Na well												
5.	2.7	23.2	12.9												
8.	1.9	4.2	14.7	33.7											
9.	1.1	1.5	7.3	30.7	10.0	20.5	1.3								
10.	1.4	0.9	0.7	1.3								14.2	10.8	13.6	
12.	1.1	0.7	0.6	0.7	1.9	5.0	22.8	9.7							
13.	0.6	0.3	0.6	1.4	1.2	2.2	4.1	20.8	21.1						
15.	2.4	2.8	1.9	2.5	2.5	2.5	5.0	3.7	5.2						37.5

Note: Intake 10 mEq. sodium per 24 hours until sodium diuresis starts.

RESULTS

It was found that portal pressure rose when the portal vein was occluded with the fingers in 6 of 9 instances in which this phenomenon was tested. In no case did the pressure fall (Table I). The measurements were usually taken in a gastroepiploic vein or branch thereof. No measurements were made of the pressure on the hepatic side of the obstruction.

Four patients were classified (Table IV) as operative deaths (a term which we extended to 60 days for this study). Three of these showed progression of liver damage and died in coma at 16, 53, and 60 days. One patient died at 56 days of a wound infection, subhepatic and subphrenic abscess, and empyema. No autopsy was permitted on C. M. The other 3 all had patent shunts at autopsy.

Failure to cure ascites by shunting occurred in 2 cases. One of the failures was a side-to-side shunt in a man who lived for 1 year and 9 months but never acquired the ability to excrete sodium adequately. He had marked anasarca during much of his last few months and finally died in another hospital of advanced liver disease and an undiagnosed intestinal disorder. No autopsy was permitted and the status of the shunt is therefore unknown. The other failure occurred in a man who lived for 9 months after an end-to-side shunt and never acquired the ability to excrete sodium. He developed recurrent ascites requiring taps and also marked edema and pleural effusion. His shunt was patent at autopsy.

There were 4 late deaths in patients whose ascites had been cured by end-to-side shunts. One man died at 5 months of what we take to have been acute fulminating homologous serum jaundice. Two died of progression of

their cirrhosis (1 year 8 months and 6 years 7 months). There was a single instance of death from massive upper gastrointestinal hemorrhage (3 years 1 month) in a man who was drinking heavily and bled from gastritis and esophagitis. Autopsy demonstrated a patent shunt and no ulcer or varices.

TABLE IV. SURVIVAL TIMES

PATIENT	TIME	COMMENTS
Operative deaths		
C. M.	Died 16 days	Progressive liver failure, jaundice, coma
M. G.	Died 53 days	Progressive liver failure, jaundice, coma
M. M.	Died 56 days	Wound infection, empyema
H. H. L.	Died 60 days	Liver failure, coma, splenorenal shunt
Failure to cure ascites		
C. C.	Died 9 mo.	Anasarca, end-to-side shunt open at autopsy
P. DeS.	Died 1 yr. 9 mo.	Anasarca, side-to-side shunt; no autopsy
*Late deaths (ascites cured)**		
A. W.	Died 5 mo.	Homologous serum jaundice (?)
M. H.	Died 1 yr. 8 mo.	C. V. A., parkinsonism, progressive cirrhosis
J. S.	Died 3 yr. 1 mo.	Bleeding gastritis; progressive cirrhosis; alcohol
L. B.	Died 6 yr. 7 mo.	Progressive cirrhosis; alcohol; terminal ascites
Surviving (February, 1960)†		
E. S.	Living 6 yr. 4 mo.	
H. L. L.	Living 5 yr. 5 mo.	
J. E.	Living 4 yr. 11 mo.	Drinking
A. B.	Living 4 yr. 8 mo.	
H. C.	Living 4 yr. 2 mo.	

*All four end-to-side shunts open at autopsy.
†All five had end-to-side shunts. None have ascites.

TABLE V. PREOPERATIVE LIVER FUNCTION TESTS

PATIENT	BILIRUBIN	CEPHALIN FLOCCULATION	A/G RATIO	BROMSULPHALEIN	PROTHROMBIN TIME	ALKALINE PHOSPHATASE (BODANSKY)
Operative deaths (60 days)						
C. M.	3.2	3	2.5/4.0	50	19 (15)	5.5
M. G.	3.8	1	2.5/1.4	45	21 (14)	4.8
M. M.	0.6	4	3.4/3.5	40	23 (15)	9.8
H. H. L.	0.6	1	4.1/2.9	30	17 (15)	12.2
Failure to cure ascites						
C. C.	1.3	1	2.5/3.0	43	18 (14)	3.9
P. DeS.	0.8	3	3.4/3.3	35	19 (15)	4.6
Ascites cures						
L. B.	0.7	0	3.7/2.4	10	21 (15)	6.2
E. S.	1.1	0	3.6/2.6	35	16 (15)	2.7
H. L. L.	0.6	1	2.8/1.9	40	18 (14)	5.4
J. E.	1.2	3	3.0/3.5	38	18 (12)	7.8
A. B.	1.3	3	2.7/4.9	42	17 (13)	28.0
H. C.	1.8	0	3.9/2.2	28	14 (14)	13.5
J. S.	1.0	3	3.4/3.5	36	18 (15)	3.9
M. H.	1.0	0	2.8/3.1	22	15 (15)	13.8
A. W.	2.4	3	2.6/4.0	45	18 (14)	6.1

The remaining 5 patients (all with end-to-side shunts) are living and well and are free of ascites. None requires salt restriction.

The time of onset of sodium diuresis was usually 3 to 4 weeks after operation in the present group of patients. In some instances the exact time of onset is not known since it occurred after discharge from the hospital (Table III).

The relationship of preoperative liver function tests to survival and cure of ascites (Table V) is perhaps of limited validity without a larger series but we were impressed that 4 of the 9 patients with "successful" shunts had 3 plus cephalin flocculation and thymol turbidity tests and that the 3 highest levels of alkaline phosphatase encountered were among the patients with "successful" results. The flocculation tests and alkaline phosphatase were thus of doubtful prognostic significance in the present series. However, it is noted that results were "successful" in 8 of the 9 patients who showed prothrombin times of 18 seconds (normal 15) or less, serum albumin of 2.7 Gm. per cent or more, Bromsulphalein retention (at 30 minutes) of 42 per cent or less, and a serum bilirubin of 1.8 mg. per cent or less. Among the 6 patients with "unsuccessful" results, 4 had prothrombin times which were worse than the one mentioned above. The same was true of 3 for albumin, 3 for Bromsulphalein, and 2 for bilirubin. These 4 tests therefore seemed to have some prognostic significance.

DISCUSSION

At the present time one can find data to support almost any view he wishes to hold on the physiology of ascites formation in cirrhosis and its resolution following shunting. The usefulness of an extra outflow tract from the congested liver is logical and is supported by the decompression which results from the retrograde flow through the upper part of the portal vein following side-to-side shunting.[29] However there is good evidence that a considerable decompression is afforded by rerouting the portal inflow away from the liver by an end-to-side shunt. Wedged hepatic vein pressures and also hepatic blood flows are found to be less after end-to-side shunting than before operation.[7, 9, 24] The existence of some portal hypertension seems necessary to the development of ascites in cirrhosis and yet there is disagreement as to the importance of different degrees of hypertension. Sometimes patients with the highest pressures do not have ascites and yet ascites can be seen in patients with relatively low pressures. In studying large groups of patients with ascites by wedged hepatic vein pressures there is conflicting evidence on the role of the pressure. In 1 series[28] pressure and ascites seemed unrelated, whereas in another,[15] there was a correlation. When measured at operation there was no correlation between ascites and pressure.[7]

It has been shown in dogs with ascites secondary to inferior vena caval constriction that the ascites resolves when portal vein constriction is added.[11] This was found to be true in spite of a normal portal pressure with vena caval obstruction. The observation suggests that cutting down inflow is effective even when outflow obstruction still exists.

In attempting to evaluate the above inconsistencies it is important to critically examine the experimental methods. This is now being done by a number of investigators. Certain obvious precautions must be followed. Patients must be tapped dry before wedged hepatic vein pressures have meaning.[15] The role of external pressure, coughing, and so on, has been assessed.[27] More subtle technical problems have also been described. Apparently the mild negative pressure applied to a hepatic vein catheter in draining a sample results in a redistribution of blood supply to that area of the liver so that the share

contributed by the hepatic artery inflow is increased.[3, 26] This makes interpretation of data on oxygen saturation, and so on, suspect where hepatic vein content is studied. An artefact of circulation is also possibly introduced when contrast media are introduced into the portal system under pressure in the performance of splenoportography. Studies of hepatic blood flow by the Bromsulphalein method must be interpreted with great care when dealing with cirrhotic livers since the disposition of the test material by the liver is partially by storage rather than exclusively by excretion into the bile. Thus the Fick principle is not as completely applicable as one might desire.

In view of the conflicts existing in the interpretation of hemodynamic data there has been an increasing interest in determining the role of liver conjugation of steroids in patients with cirrhosis.[21, 22] Lest this approach push aside continuing research on hemodynamics, it should be mentioned that in our postshunt patients dying of acute liver deaths (hepatitis) or slow, increasing liver failure we have not observed the reaccumulation of ascites.

Perhaps there has been too much stress on the study of the site and factors governing the secretion of ascitic fluid. It is known that free fluid in serous cavities has a rapid turnover rate,[5, 23] the rate being 40 to 80 per cent per hour in the case of ascitic fluid.[23] While it is now felt that fluid enters the peritoneal cavity largely from the liver surface, it has not been determined from which surface it leaves. It is possible that the disturbance in portal hypertension is one of difficult reabsorption rather than increased secretion of fluid. If the site of reabsorption were from splanchnic surfaces rather than the liver surface, the emphasis would then be refocused on the prehepatic portal hypertension and its relief rather than the intrahepatic portal hypertension. Even more likely than any of the above, however, is the possibility that a very delicate and slight imbalance between entry and exit of ascitic fluid exists in the patient with cirrhosis and this balance is not exclusively a matter of either intra- or prehepatic portal hypertension. It is quite likely, in fact, that the relative importance of pressures in the several areas concerned is highly variable with the stage of the disease and is also variable from minute to minute in the same patient.

Solution to the question of the usefulness of shunts in treating ascites and of the comparative value of different types of shunts may come only from the collection of data on large clinical series of patients in whom the shunts have been established. We feel from our own studies and those of others that either an end-to-side or a side-to-side shunt will cure ascites. The question which seems to remain is whether shunting is detrimental or beneficial to liver function and what, if any, differences may exist in these regards between end-to-side and side-to-side shunts.

SUMMARY

1. Fifteen patients with cirrhosis of the liver and "medically irreversible" ascites have been subjected to portacaval shunting. Thirteen underwent end-to-side portal vein to vena cava shunts, 1 a side-to-side shunt, and 1 a splenorenal shunt.

2. All were subjected to careful in-patient studies and preparation for at least 1 month preoperatively and throughout their entire in-hospital postoperative course. This included balance studies with daily urinary output of sodium being stressed in the present report.

3. These patients constituted a high risk series from the standpoint of liver function and 4 did not live beyond 60 days. Failure to cure ascites occurred in 2 instances. One of these followed a side-to-side shunt.

4. Nine patients were considered to have experienced successfully cured ascites and 5 of these patients are still living at 4 to 6 years after operation completely free of ascites. All 9 had undergone end-to-side shunts.

5. It is concluded that end-to-side portacaval shunting cures ascites in cirrhosis of the liver.

We are indebted to Dr. Arthur H. Blakemore and Dr. George H. Humphreys, II, for advice, encouragement, and guidance. Many of the shunts listed were performed by or with the help of Dr. Blakemore. We wish to thank Dr. David V. Habif, under whose direction the metabolic studies were carried out on the first 3 patients. Dr. Arthur B. Voorhees, Jr., has seen some of the patients at follow-up visits. The surgical house staff and the nursing, dietetic, and technical staff of the surgical metabolic unit have also been of immeasurable help.

REFERENCES

1. Baggenstoss, A. H., and Wollaeger, E. E.: Portal Hypertension Due to Chronic Occlusion of the Extrahepatic Portion of the Portal Vein: Its Relation to Ascites, Am. J. Med. **21**: 16, 1956.
2. Blakemore, A. H.: Personal communication.
3. Brauer, R. W., Schill, O. S., and Krebs, J. S.: Studies Concerning Functional Differences Between Liver Regions Supplied by the Hepatic Artery and by the Portal Vein, J. Clin. Invest. **38**: 2211, 1959.
4. Child, C. G., III, and Donovan, A. J.: Current Problems in Management of Patients With Portal Hypertension, J. A. M. A. **163**: 1219, 1957.
5. Clauss, R. H., Yacoubian, H., and Barker, H. G.: Dynamics of Pleural Effusions, S. Forum **7**: 201, 1957.
6. Eisenmenger, W. J., and Nickel, W. F.: Relationship of Portal Hypertension to Ascites in Laennec's Cirrhosis, Am. J. Med. **20**: 879, 1956.
7. Ekman, C. A.: Portal Hypertension; Diagnosis and Surgical Treatment, Acta chir. scandinav., Suppl. 222, 1957.
8. Freeman, S.: Recent Progress in the Physiology and Biochemistry of the Liver, M. Clin. North America **37**: 109, 1953.
9. Gliedman, M. L., Sellers, R. D., Grant, R. N., Burkle, J. S., Leevy, C. M., and Vestol, B. L.: Observations on the Effects of Portacaval Anastomosis Upon the Hemodynamics of the Liver, Surg. Gynec. & Obst. **108**: 223, 1959.
10. Habif, D. V., Randall, H. T., and Soroff, H. S.: The Management of Cirrhosis of the Liver and Ascites With Particular Reference to the Portacaval Shunt Operation, SURGERY **34**: 580, 1953.
11. Hayano, S.: Experimental Studies on Ascites, Arch. f. Japanische chir. **27**: 1063, 1958.
12. Hume, D. M.: In discussion of paper by Long, T. L., Lombardo, C. R., and Morrow, A. G.: SURGERY **47**: 125, 1960.
13. Hyatt, R. E., and Smith, J. R.: Mechanism of Ascites, Am. J. Med. **16**: 434, 1954.
14. Kelty, R. H., Baggenstoss, A. H., and Butt, H. R.: The Relation of the Regenerated Liver Nodule to the Vascular Bed in Cirrhosis, Gastroenterology **15**: 285, 1950.
15. Krook, H.: Circulatory Studies in Liver Cirrhosis, Acta med. scandinav. Suppl. 318, 1956.
16. Large, A. M., and Johnston, C. G.: The Partial or Complete Portacaval Shunt, Surg. Gynec. & Obst. **109**: 107, 1959.
17. Longmire, W. P., Jr., Mulder, D. G., Mahoney, P. S., and Mellinkoff, S. W.: Side-to-Side Portacaval Anastomosis for Portal Hypertension, Ann. Surg. **147**: 881, 1958.
18. Madden, J. L., Lore, J. M., Jr., Gerald, F. P., and Ravid, J. M.: The Pathogenesis of Ascites and a Consideration of Its Treatment, Surg. Gynec. & Obst. **99**: 385, 1954.
19. Mallet-Guy, P., Devic, G., Feroldi, J., and Desjacques, P.: Étude expérimentale des ascites; sténoses veineuses post-hépatiques et transposition du foie dans le thorax, Lyon chir. **49**: 153, 1954.

20. McDermott, W. V.: The Treatment of Cirrhotic Ascites by Combined Hepatic and Portal Decompression, New England J. Med. **259**: 897, 1958.
21. Mittelman, A., Gore, D. O., and Barker, H. G.: Urinary Aldosterone Excretion Before and After Portacaval Shunting in Cirrhosis of the Liver, Fed. Proc. **18**: 105, 1959.
22. Mittelman, A., Gore, D. O., and Barker, H. G.: Urinary Aldosterone Excretion Before and After Portacaval Shunt, S. Forum **10**: 346, 1960.
23. Prentice, T. C., Siri, W., and Joiner, E. E.: Quantitative Studies of Ascitic Fluid Circulation With Tritium-labelled Water, Am. J. Med. **13**: 668, 1952.
24. Redeker, A. G., Geller, H. M., and Reynolds, T. B.: Hepatic Wedge Pressure, Blood Flow, Vascular Resistance and Oxygen Consumption in Cirrhosis Before and After End-to-Side Shut, J. Clin. Invest. **37**: 606, 1958.
25. Reemtsma, K., Carlson, R. E., Habif, D. V., and Barker, H. G.: Changes in Sodium Metabolism Following Portacaval Shunts in Patients With Cirrhosis and Ascites, S. Forum **7**: 422, 1957.
26. Sapirstein, L. A., and Reininger, E. J.: Catheter-Induced Error in Hepatic Venous Sampling, Circulation Res. **4**: 493, 1956.
27. Taylor, F. W.: Portal Tension and Its Dependence on External Pressure, Ann. Surg. **140**: 652, 1954.
28. Taylor, W. J., and Myers, J. D.: Occlusive Hepatic Venous Catheterization in the Study of the Normal Liver, Cirrhosis of the Liver, and Non-cirrhotic Portal Hypertension, Circulation **13**: 368, 1956.
29. Warren, W. D., and Muller, W. H.: A Clarification of Some Hemodynamic Changes in Cirrhosis and Their Surgical Significance, Ann. Surg. **150**: 413, 1959.
30. Welch, C. S., Attarian, E., and Welch, H. F.: The Treatment of Ascites by Side-to-Side Portacaval Shunt, Bull. New York Acad. Med. **34**: 249, 1958.
31. Welch, C. S., Welch, H. F., and Carter, J. H.: The Treatment of Ascites by Side-to-Side Portacaval Shunt, Ann. Surg. **150**: 428, 1959.

Discussion

(Papers by Chapman, Goldsworthy, Nyhus, Volwiler, and Harkins; Bono, Moreno, Rousselot, and Panke; Barker and Reemtsma)

DR. BEN EISEMAN (Denver, Colo.).—I would like to make 3 short points. The first is that I think only those of us who have been interested in liver perfusion and making liver perfusion grafts in the laboratory can appreciate the sophisticated nature of this very humbly presented paper. To obtain such reproducible results in liver perfusion is something that comes only with the years of practice that the people in Seattle have had, and I might note that they have been extremely careful in reporting anything at all, waiting until they have exact data. It is an extremely sophisticated subject. Second, I think that this type of study puts a large spike in the lid of the coffin of Andrews' extremely attractive theory in which he drew the analogy between the liver where there is a liver cell and a bile duct, and the kidney where there is a glomerulus and a tubule. It was an extremely attractive theory and Andrews' data from dogs would make us think that the bile ducts might act as tubules, but I think that the very careful data given suggest that this is not the case. I might point out, as I am certain Dr. Harkins, Dr. Chapman, and others note, that Dr. Andrews' data was from another species which they know, better than I, acts very differently as far as liver perfusion is concerned than do the calves. The third point is that I think I would like to predict that the development of more perfect techniques of liver perfusion and perhaps liver grafting have real clinical importance. It is along this particular line that our interest lies. Patients in hepatic coma are often in a much more favorable condition to survive advanced coma than analogous patients in renal failure because, of course, the liver can regenerate so much better than can the kidney. If you can tide them over an acute episode of coma, patients with hepatic coma frequently can improve their hepatic function much more than can analogous patients with severe renal failure. Most of us have had high hopes for drugs that lowered blood ammonia, but I think we have been disappointed in this. I believe that in the techniques such as Dr. Volwiler and Dr. Harkins and Dr. Chapman and his colleagues, both in the Department of Medicine and the Department of Surgery at Seattle, have demonstrated to us today lie the real hope in the treatment of patients with advanced attacks of coma.

DR. CHARLES G. CHILD, III (Ann Arbor, Mich.).—As many of you know, my associates and I have been laboring long and hard to produce portal hypertension and esophagogastric varices in laboratory animals. We have found that a variety of hepatic obstructions will force blood past the liver and open up collaterals similar to those which have been so well demonstrated by Dr. Bono this morning. I have one question that I should like to ask—have the varices produced by the authors been submucosal?

My question is prompted by our most recent efforts in which we have applied Harold Laufman's technique (Surg. Gynec. & Obst., 110: 451-456, 1960) to the dog. We too have produced in this animal a wide variety of dilated channels about the esophagus. Unfortunately none of these are submucosal. They all are located intramurally or extramurally. To reproduce varices as they occur in man must be our objective. To provide a maximally useful experimental animal the varices should be submucosal.

I should like, therefore, to ask Dr. Bono whether he has visualized the esophagus in his animals to determine whether his varices are submucosal.

GEORGE L. NARDI (Boston, Mass.).—I would like to comment briefly and also say a few words in Dr. McDermott's absence.

Dr. McDermott and Dr. Kestens, in our surgical department, have worked out a very nice perfusion technique for the dog liver that has permitted us to carry out metabolic studies in vivo and in vitro, if you will. After prolonged in vivo perfusions with complete isolation of the liver the animal has survived and studies with Bromsulphalein, radioactive metabolites, and colloidal suspension have shown normal biliary excretion, metabolism, and flow.

As a result of our studies of hepatic blood flow in man we would agree with Dr. Childs. The flow through varices in patients would seem to be quite high, about 400 to 500 c.c. per minute. We have also found that obstruction of the portal vein at the time of surgery results in little acute change in liver blood flow, which may be a reason why these are accomplished so readily.

We can also confirm the good results reported by Drs. Barker and Reemtsma. Satisfactory relief of ascites has resulted after both the double-barreled shunt of McDermott as well as side-to-side and end-to-side portacaval shunts.

DR. ALLAN D. CALLOW (Boston, Mass.).—I should like to ask the Presbyterian group if they have had any experience with the antialdosterone compounds in those few patients they reported who did not respond with relief of ascites following the shunt. We have lost some enthusiasm for surgical relief of ascites since these compounds were introduced.

DR. MARVIN M. NACHLAS (Baltimore, Md.).—Dr. Rousselot and his associates are to be congratulated for the excellent and informative splenoportograms which they presented. I have always enjoyed Dr. Rousselot's experimental work, even though I cannot agree with his clinical evaluations concerning benefits derived from portacaval shunts. The great interest displayed in the problems of portal hypertension during the past 20 years arose primarily from the activities of Dr. Rousselot, who was the first surgeon to put a needle into the portal vein, and demonstrate conclusively that portal hypertension exists in man.

Drs. Barker and Reemtsma have reported on the relief of intractable ascites by the performance of portacaval shunts in a small number of patients. The reason for their success is difficult to understand, since there are at least 4 and possibly 5 factors concerned with the production of ascites in patients with cirrhosis of the liver, only 1 of which would be affected directly by creation of a shunt. These are: (1) lowered serum albumin, (2) decreased sodium excretion, (3) the antidiuretic substance of Ralli, (4) portal hypertension, and (5) outflow tract obstruction with liver weeping, as described by Madden. Theoretically, if patients could be selected in which 4 of these factors were nearly within normal limits and the prominent abnormality associated with the ascites was the portal hypertension, then one could understand (perhaps even expect) good results from a portacaval shunt. I doubt that Drs. Barker and Reemtsma were able to find patients where portal hypertension alone produced the intractable ascites, although not having read the manuscript, I cannot be certain about this.

Why the correction of only one among several causes of ascites should eliminate the ascites is most mysterious. I think that perhaps the greatest value which may be derived from their report will not be that many patients with ascites will be cured by portacaval shunt, but rather that the stimulus to solve another unknown may lead them or others to obtain more information concerning the factors responsible for the intractability of ascites in some patients with cirrhosis of the liver.

DR. CHAPMAN (closing).—I would like to thank Drs. Eiseman and Nardi for their comments and point out that we should always bear in mind the fact that species differences do occur and may account for different results by different investigators. The calf has a liver that does not possess a normal bacterial flora; the canine liver, in contrast, does. The experiments reported here this morning were performed in sterile livers. In addition, the bovine liver (unlike the canine liver) is not an anaphylactoid end-organ and in our laboratory has been less difficult to study than the canine liver.

DR. MORENO (closing).—On behalf of the members of the group, I would like to thank the discussors, Dr. Child, Dr. Nardi, and Dr. Nachlas, for their nice comments. In answer to Dr. Child, I will say that these varicose veins we have shown you in the esophagus were not located in the submucosal layer. We believe that perhaps we never see these dilated veins in the submucosal layer of the esophagus of the rat because there are very few veins in the submucosal layer. We just try to demonstrate the similarity in the basic mechanisms by which the hepatic bypass was accomplished in these animals, in producing a hepatic situation similar to the one that we observed in cirrhotic human patients.

DR. BARKER (closing).—I would like to thank the discussors and answer the question about antialdosterone compounds. We have used these substances and found them helpful at times, particularly in the milder ascites. We have never advocated and do not now advocate the use of the portacaval shunt operation as a treatment for mild ascites. Obviously it will oftentimes be performed on patients who have mild ascites but where another indication exists, namely, hemorrhage from varices. The patients reported upon today all suffered severe ascites and most did not respond well to any diuretic or to antialdosterone compounds.

LONG ESOPHAGOMYOTOMY FOR DIFFUSE SPASM OF THE ESOPHAGUS AND HYPERTENSIVE GASTROESOPHAGEAL SPHINCTER

F. HENRY ELLIS, JR., M.D., CHARLES F. CODE, M.D., AND ARTHUR M. OLSEN, M.D., ROCHESTER, MINN.

(From the Sections of Surgery, Physiology, and Medicine, Mayo Clinic and Mayo Foundation)

SURGICAL measures are assuming an increasingly important role in the management of benign esophageal obstruction. As an example, indications for the use of esophagomyotomy (the modified Heller procedure) in the treatment of achalasia of the esophagus have been extended. Increasing knowledge of normal and abnormal esophageal function has been primarily responsible for this trend.

Precise diagnosis is essential for proper surgical treatment of esophageal disorders. Accurate information may be gained through the use of studies of esophageal motility,[3, 13] which have been performed on patients with a variety of esophageal disorders,[6-8, 17] as well as on patients with achalasia of the esophagus before and after esophagomyotomy.[18] The findings have suggested that certain disorders characterized by hyperactivity of the esophagus and its sphincter might be benefited by a more extensive esophagomyotomy than that originally proposed by Heller for the treatment of esophageal achalasia.

As a result, we have employed an extended esophagomyotomy in 20 patients with functional disorders other than achalasia. Fourteen of these patients had diffuse spasm of the esophagus, 4 had diffuse spasm and a hypertensive gastroesophageal sphincter, and the remaining 2 patients had only a hypertensive sphincter.

DEFINITIONS AND CLINICAL PICTURES

Before proceeding with an analysis of the clinical data, it is essential not only to define clearly the nature of these physiologic disorders, but also to compare them with the normal state and with other disease states. It should be emphasized that the cause of these conditions is unknown.

Diffuse Spasm of the Esophagus.—This disease appears in the literature under a variety of other terms, including "pseudodiverticulosis," "functional diverticula," "segmental spasms," and "curling" or "corkscrew" esophagus. Osgood[19] (1889) is given credit for the first description of diffuse esophagospasm. Although others may have recognized it in subsequent years, it remained for Moersch and Camp[16] to describe it as a clinical entity. Their description is a classic one and is as applicable today as it was originally.

Pain and dysphagia are the predominant symptoms, with pain being more pronounced and dysphagia occurring intermittently or not at all. The pain varies from a sensation of discomfort beneath the lower half of the sternum to severe, colicky, substernal pain extending through to the back, between the tips of the scapulas, and into the neck or shoulders and

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The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

arms. It may mimic cardiac pain. Eating may provoke an attack of pain or it may come on spontaneously and may even awaken the patient at night. Patients with this disease tend to be high-strung and nervous, and the diagnosis of psychoneurosis may be entertained. The symptoms are intermittent and are more likely to be troublesome than incapacitating. Even in an attack, the patient seldom appears seriously ill.

It is important to differentiate this condition from achalasia of the esophagus. Achalasia is synonymous with the term "cardiospasm," and it denotes a condition characterized by motor failure of the esophagus associated with an absence of relaxation of the lower sphincter.

Descriptions of the roentgenographic appearance of the esophagus in diffuse spasm are numerous[11, 14, 16] and tend to be in general agreement. Examination after the ingestion of barium reveals distortion of the lower half or third of the esophagus, characterized by spasm. All variations of spasm may be seen, ranging from simple narrowing to segmental spasm and finally to the extreme changes of pseudodiverticulosis with complete segmentation of the esophagus into pockets, producing a beaded appearance (Fig. 1). Johnstone[14] commented on the frequently associated finding of a small diaphragmatic hernia.

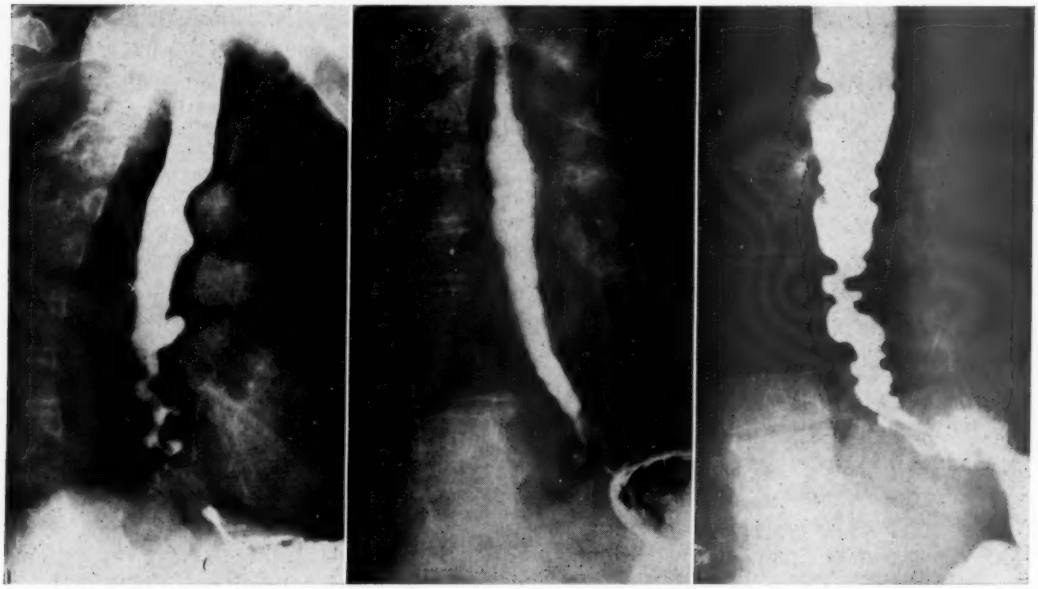


Fig. 1.—Roentgenograms from 3 different patients with diffuse esophagospasm. Note the varied appearance. (Reproduced with permission of the publisher and authors from Olsen, A. M., Harrington, S. W., Moersch, H. J., and Andersen, H. A.: J. Thoracic Surg. 22: 164, 1951.)

The roentgenographic findings in diffuse esophagospasm may be simulated by the irregular contractions sometimes seen in older people. These are asymptomatic and probably represent the tertiary contractions described by Templeton. It has been suggested that the terms "curling" and "corkscrew" esophagus be reserved for these cases. Likewise, roentgenograms of the esophagus after successful treatment for achalasia may closely resemble those made in instances of diffuse spasm but the patients may be asymptomatic.

Prior to the availability of studies of esophageal motility, the clinician was forced to rely heavily on the radiologist for support of his clinical impression. Creamer and associates,[7] however, pointed out that many patients with abnormal motility records, characteristic of diffuse spasm of the esophagus, may have a normal esophageal roentgenogram, which emphasizes the importance of studying esophageal motility in patients suspected clinically of having this condition.

A specific abnormal pattern of the deglutitive pressures is present in patients with diffuse spasm.[7, 17] The primary peristaltic wave usually can be recognized over the upper half or two-thirds of the esophagus. In the lower half or third, the peristaltic front of the

deglutitive response is lost and is replaced by simultaneous and sometimes repetitive and prolonged contractions that are often of great amplitude (Figs. 2 and 3). Most patients do not display any abnormality of the 2 sphincters (Fig. 4), although occasionally there may be poor relaxation of the inferior esophageal sphincter. Evidence of associated hiatal herniation is frequently present, particularly in patients whose esophageal roentgenograms are diagnostic of diffuse esophagospasm.

Hypertensive Gastroesophageal Sphincter.—This condition has been described only recently.[4] It is possible, of course, that some of the cases reported in the literature as examples of "idiopathic muscular hypertrophy of the esophagus" may represent this condition.[20, 22] It is more likely, however, that such cases were those of diffuse spasm of the esophagus.

An analysis of esophageal motility in patients with hypertensive gastroesophageal sphincter recently has been completed.[4] In these patients, pressures in the inferior sphincter were excessive, being more than 140 cm. of water as detected by balloon transducers (Fig. 5), and there was usually an associated poor relaxation of the sphincter. Motility in the body of the esophagus was sometimes normal, although some elements of the picture of diffuse spasm were present in the majority.

SURGICAL TREATMENT

The great majority of patients with the conditions under discussion are not greatly troubled by their symptoms, so that treatment is rarely necessary. When pain is prominent, treatment may be required, particularly when the pain is severe and attacks are frequent. Medical treatment, including esophageal dilation, has been disappointing.[16, 21] Psychiatric therapy has been equally unrewarding.

When one reviews the literature, it is extremely difficult to know whether surgical treatment for diffuse spasm of the esophagus or for hypertensive gastroesophageal sphincter has been employed, since data on esophageal motility are not available. For instance, it may well be that some of the patients classified by Sweet[23] as having type II achalasia actually may have had diffuse spasm of the esophagus. These patients complained of pain and presented little roentgenographic evidence of esophageal dilatation; hypertrophy of the distal esophageal musculature was found at operation. They were benefited by esophagomyotomy.

Allison performed bilateral vagotomy and, later, esophagectomy on one of Sloper's patients who had muscular hypertrophy of the esophagus. Recently, several patients with idiopathic muscular hypertrophy of the esophagus have been treated by extensive submucosal esophagomyotomy, with good results.[2, 15]

REVIEW OF PATIENTS TREATED AT THE MAYO CLINIC

Between July, 1956, and January, 1960, a total of 20 patients have undergone extended esophagomyotomy at the Mayo Clinic by the technique to be described. As previously stated, 14 patients had diffuse spasm of the esophagus, 4 had diffuse spasm of the esophagus and a hypertensive gastroesophageal sphincter, and 2 had only a hypertensive sphincter. The diagnosis was made on the basis of studies of esophageal motility in all instances. Fourteen were women and 6 were men; their ages ranged from 29 to 71 years, averaging 49.

Symptoms.—With few exceptions, symptoms had been present for prolonged periods, averaging 39 months, with a range from 2 weeks to 10 years. Surgical treatment, particularly in the early periods of the study, was reserved for patients who had severe, persistent, and incapacitating symptoms, although the indications have been liberalized in recent months because of satisfaction with the early results.

Pain and dysphagia were the commonest symptoms, pain being the more frequent and prominent of the two (Table I). Eighteen of the 20 patients experienced pain and in 6 patients, it was the only symptom. The character of the pain was variable but similar to that originally described by Moersch and Camp, as already detailed. In 2 instances, a diagnosis of coronary insufficiency had been entertained in the past. Anxiety and nervous

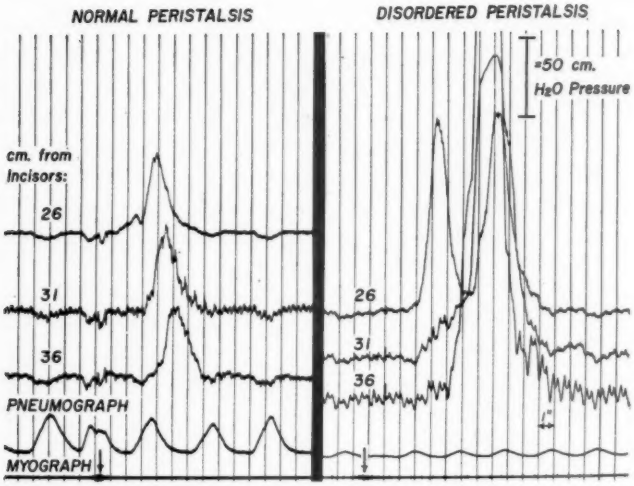


Fig. 2.—Deglutitive pressures in lower half of esophagus in healthy person contrasted with those in patient with diffuse esophagospasm. Note the loss of the sequential pattern in the record to the right (from a patient with diffuse spasm). Two contractions occurred at the 26 cm. level, the second of which coincided with the contractions at the 31 and 36 cm. levels. The pressures achieved were also abnormally high. (Reproduced with permission of the publisher and authors from Creamer and associates.[7])

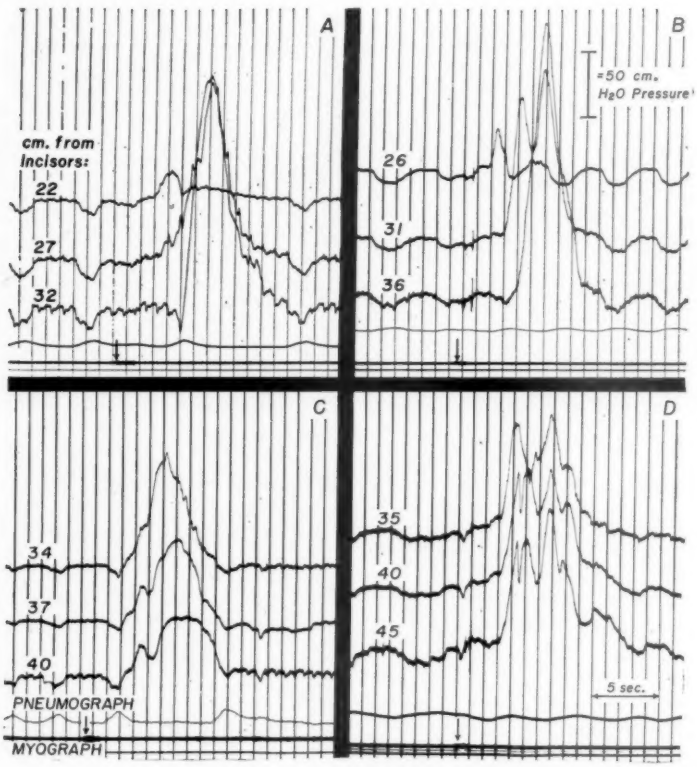
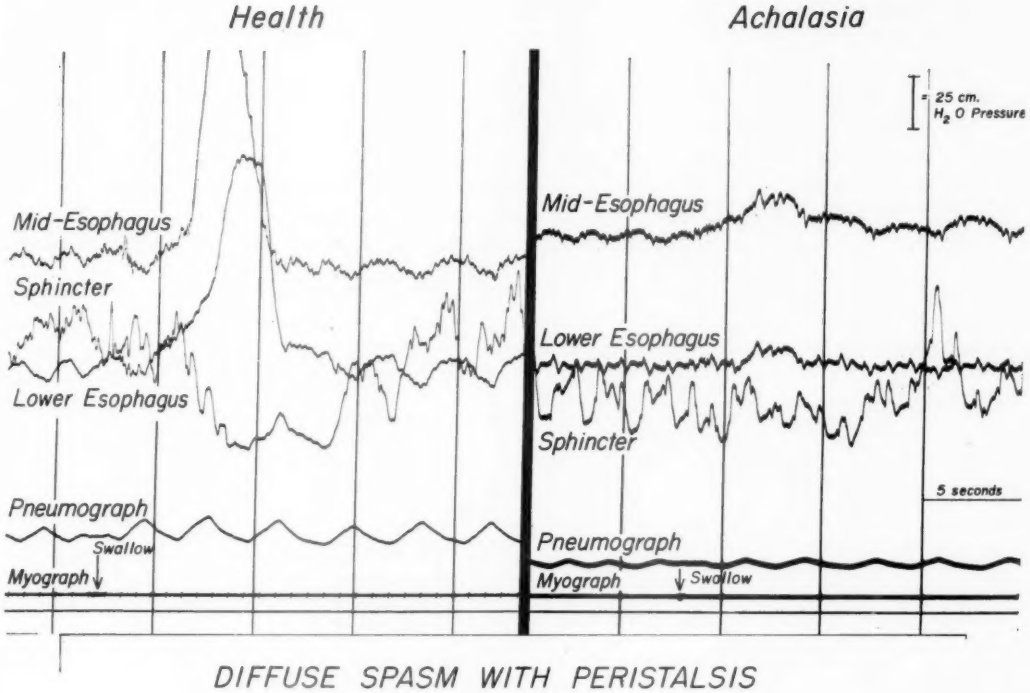


Fig. 3.—Deglutitive pressures in 4 patients with diffuse esophagospasm, showing variations in abnormal response. Note the simultaneous peaks in each tracing, and the simultaneous onset of the deglutitive response in *C* and *D*. The abnormally high pressures, the repetitive contractions, and their prolonged duration are all apparent. (Reproduced with permission of the publisher and authors from Creamer and associates.[7])



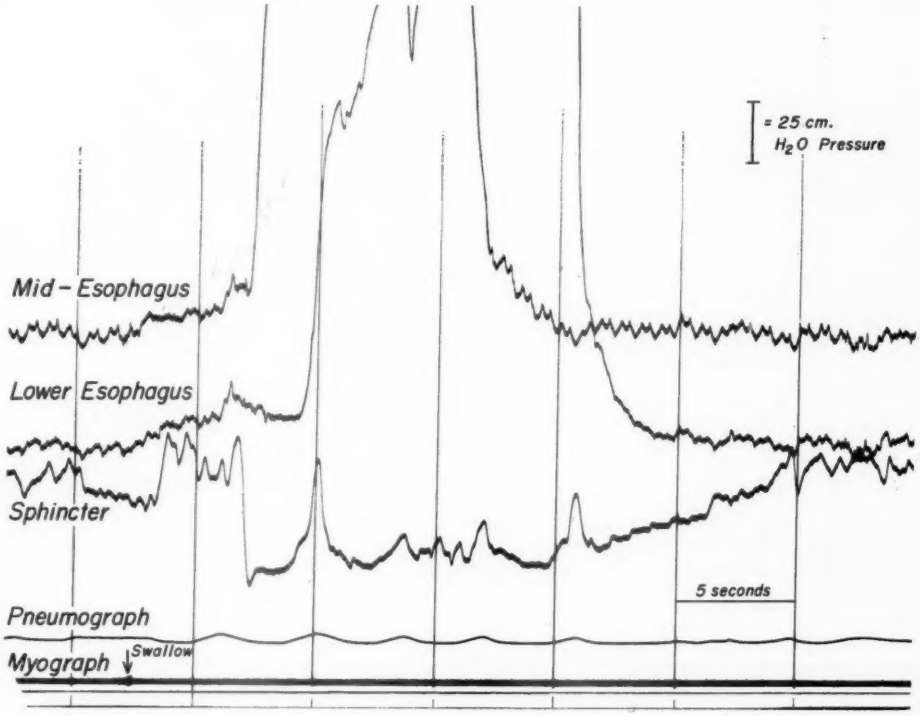


Fig. 4.—Deglutitive responses in body and lower sphincter of esophagus in health *(upper left)*, achalasia *(upper right)*, and diffuse spasm *(below)*. The downward-pointing arrows indicate swallows. Note normal peristalsis and sphincteric relaxation in health, absence of both peristalsis and sphincteric relaxation in achalasia, and excessive and repetitive contractions of long duration with sphincteric relaxation in diffuse spasm.

TABLE I. MAIN SYMPTOMS IN HYPERACTIVITY OF THE ESOPHAGUS AND ITS SPHINCTER

CONDITION	PATIENTS	SYMPTOMS		
		PAIN ONLY	PAIN AND DYSPHAGIA	DYSPHAGIA ONLY
Diffuse spasm	14	3	10	1
Diffuse spasm and hypertensive sphincter	4	2	1	1
Hypertensive sphincter only	2	1	1	0
Totals	20	6	12	2

fatigue occasionally precipitated or aggravated the symptoms. These features, coupled with a lack of objective findings from ordinary studies, led to a diagnosis of psychoneurosis in some patients, 3 of whom were actually under a psychiatrist's care.

Dysphagia, when present, more often accompanied the ingestion of solids than it did the intake of liquids, in contrast to what is noted by some patients with achalasia of the esophagus. Dysphagia occasionally was severe, and 9 patients had undergone dilation on one

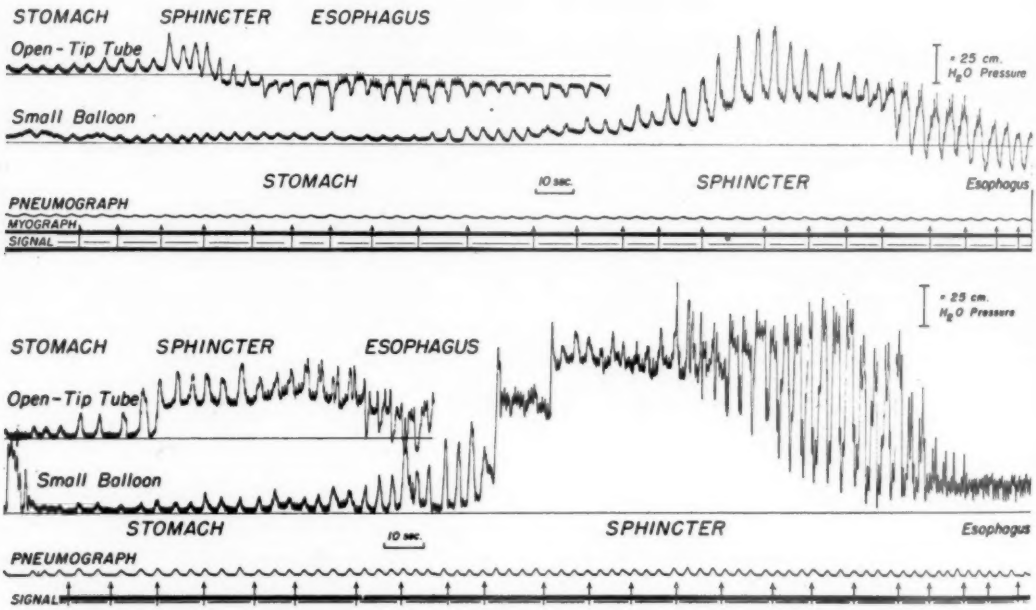


Fig. 5.—Pressure profile of the gastroesophageal sphincter in normal person *(above)* and in patient with hypertensive gastroesophageal sphincter *(below)*. Note the excessive pressures at the sphincter in the patient with a hypertensive sphincter.

or more occasions prior to operation. Other symptoms included loss of weight in 8 patients, heartburn in 6, and regurgitation or vomiting in 6. Hematemesis had occurred in 1 patient. Striking differences in the symptom complex were not apparent when the patients were subdivided into separate groups according to the findings obtained by studies of esophageal motility.

Ancillary Studies.—A diagnosis of diffuse esophagospasm was made by the radiologist in only 4 cases on the basis of the previously mentioned findings. Small diaphragmatic hernias were seen roentgenoscopically in 5 patients, 2 of whom also had evidence of diffuse spasm (Fig. 6, *a*) and 2 esophageal diverticula were noted in 1 patient (Fig. 6, *b*). Only 2 patients had any evidence of esophageal dilatation (Fig. 6, *a*). In 10 patients, esophageal roentgenoscopy did not reveal any abnormalities.

Esophagoscopy was performed on a number of patients. Other than the presence of diaphragmatic hernias in 5 patients, with some associated esophagitis of mild to moderate degree, the findings were nonspecific.

Standard diagnostic tests of esophageal motility were performed preoperatively on the 20 patients.[5-7] The tests were repeated at least once postoperatively on 19 of the 20 patients. Fifteen of these 19 patients had their first postoperative tests 6 to 21 days after operation, whereas the other 4 had their first tests later. Some patients have been studied repeatedly for 3 years after operation. In all but 2 of the studies, a balloon-covered transducer and 2 or 3 open-tipped tubes were employed. In the other 2 patients, 3 open-tipped tubes were used. The balloon and the open tips were separated by 5 cm. Each test included measurement of the resting pressures in the esophageal sphincters by withdrawal through the sphincter and the determination of the responses of the sphincters and the esophagus to swallowing. The length and pressure of the sphincter presented in this report are those obtained with the balloon-covered transducer. Both open-tip and balloon methods of recording were used to estimate changes in the motor pattern of the esophagus. Detailed analysis of these studies will be described with the results of operation.

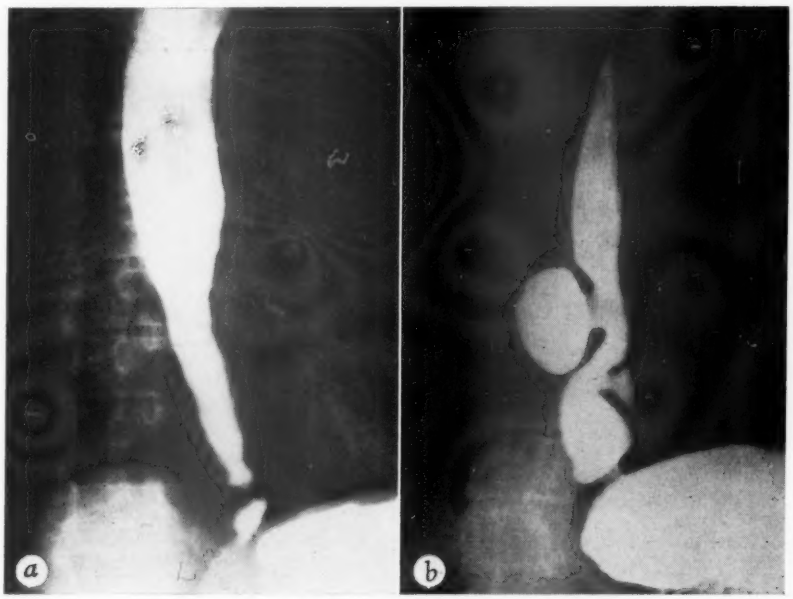


Fig. 6.—Esophageal roentgenograms from 2 patients with diffuse esophagospasm. A small diaphragmatic hernia is present in one patient *(a)*, and 2 diverticular are present in the other *(b)*.

SURGICAL TECHNIQUE

The distal portion of the esophagus was approached through the left side of the thorax in every instance, the incision being made through the seventh intercostal space or through the bed of the nonresected eighth rib. The mediastinal pleura was opened, and the esophagus was mobilized. In most respects, the operative procedure was identical to that performed by us for achalasia of the esophagus.[10] An esophagomyotomy was performed, with complete incision of the distal esophageal musculature, so that the underlying mucosa was allowed to bulge freely (Fig. 7). The incision was carried onto the stomach for a short distance (1 to 2 cm.) to ensure complete division of the distal esophageal musculature. The proximal extent of the incision varied, depending on the preoperative estimate of the length of the diseased portion of the esophagus. Grossly, the esophagus did not appear abnormal in the majority of our patients, although the muscle was abnormally thickened in 8 instances. Data from the tests of esophageal motility were utilized to estimate the extent of the disease, and the length of the esophageal incision was determined accordingly. The proximal limit of the incision in our 20 patients varied from a point on the esophagus opposite the inferior pulmonary vein to a point just below the aortic arch, the total length ranging from 8 to 15

cm. An incision of adequate length is most important. After the incision was completed, the muscle layer was dissected laterally from the mucosa to free it thoroughly from encircling fibers.

As in patients operated on for achalasia of the esophagus, attention was paid to the status of the esophageal hiatus. If it appeared to be widened, it was narrowed by sutures placed posteriorly in the crus. In contrast to cases of achalasia of the esophagus, however, a small diaphragmatic hernia frequently is associated with a hyperactive esophagus, and its repair becomes an integral part of the procedure. In all likelihood, these hernias are secondary to the primary esophageal disease. As noted previously, such hernias were detected roentgenoscopically in 5 instances. At the time of operation, similar hernias were encountered in 2 additional patients, so that 7 patients required repair of such complications. The method of Allison was employed for this purpose.

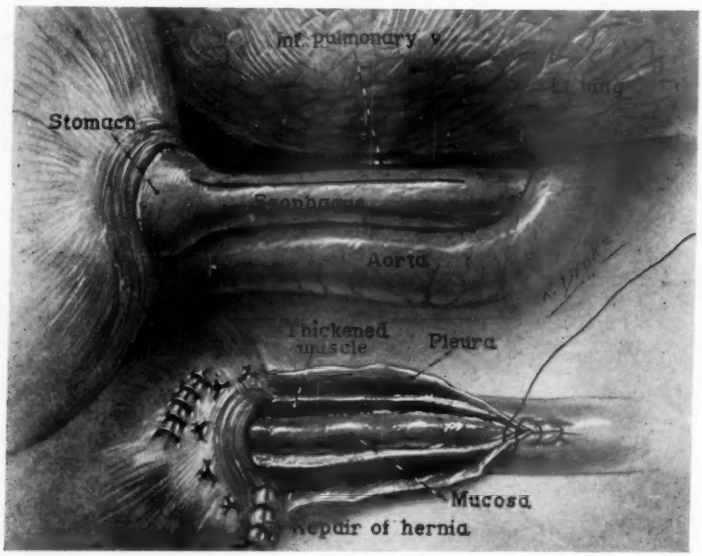


Fig. 7.—Operative procedure. A long esophagomyotomy is made, completely incising the distal esophageal musculature. The diaphragmatic hernia so often present is repaired.

The thorax was closed in the usual fashion. An intercostal tube, which was left for postoperative drainage, was usually removed on the following day. Oral feedings were permitted the evening of the operation, and the patients usually left the hospital within 1 week after the operation.

RESULTS

There were no hospital deaths or late deaths, and all patients recovered from the operation uneventfully.

Clinical Data.—Follow-up data were available on 19 of the 20 patients. In 14 instances, this was based on direct questioning and re-examination of the patient, whereas the information in 5 instances was obtained by letter. The follow-up period ranged from 6 to 35 months, averaging 15 months.

The same criteria were used that we have employed previously in evaluating the results of esophagomyotomy in the treatment of achalasia of the esophagus.[10] To be considered as having excellent results, patients must be asymptomatic for all practical purposes. Good results imply complete relief of symptoms except on rare occasions of stress or when eating with undue rapidity. Patients who are improved but who still complain of some pain,

dysphagia, or heartburn are classified as having only fair results. Patients who are unimproved or who have required postoperative dilation are considered as having poor results. Admittedly, these are strict criteria, for some patients have remained well after 1 postoperative dilation.

The results are summarized in Table II. The condition in 16 patients (84 per cent) was improved by the operation. Eight of these patients had excellent results, 7 had good results, and 1 had fair results. The results were

TABLE II. RESULTS OF LONG ESOPHAGOMYOTOMY*

INDICATION FOR OPERATION	RESULTS			
	EXCELLENT	GOOD	FAIR	POOR
Diffuse spasm	4	6	1	3
Diffuse spasm and hypertensive sphincter	3	1	0	0
Hypertensive sphincter only	1	0	0	0
Totals	8	7	1	3

*Based on 19 patients with follow-up data.

poor for 3 patients, all of whom were in the group with diffuse spasm of the esophagus alone. One of these 3 patients had a preoperative stricture and required passage of a sound after operation. In all likelihood, the diffuse spasm was secondary to an organic stricture that had not been relieved by operation. The second patient required 1 postoperative dilation but is now well and the third patient still complains of the same pain that troubled her preoperatively. The esophagomyotomy in this last patient was a short one, and it is possible that in this case an incomplete operation was performed. There was a high incidence of persistent incisional pain, 6 patients being so afflicted. Rhizotomy was required for relief of pain in 1.

Roentgenographic Data.—Postoperative roentgenograms of the esophagus and stomach were made in 15 of the 19 patients on whom follow-up data were available. It was often easy to identify the site of the esophagomyotomy (Fig. 8). Competency of the esophagogastric junction was studied in 8 patients and in only 1 was any reflux noted.

Esophageal Motility.—

Deglutitive responses in the esophagus: The pressures that developed in the incised portion of the esophagus during swallowing were decreased by the myotomy in every patient tested, and this decrease remained permanent in those patients on whom repeated tests were made. In almost all of the patients, a reduction in the duration of the increased pressure during deglutition followed the myotomy, and this change also was permanent. In some of the patients, peristaltic contractions had been recorded from the incised region before the operation. These disappeared permanently from the incised segment. Repetitive contractions, which occurred commonly before operation, disappeared almost entirely from the incised zone after operation. In general, the amplitude, duration, and repetitiveness of the deglutitive responses observed preoperatively in the incised region were reduced, and this change was accompanied by the establishment of permanently simultaneous and rather feeble responses to swallowing (Fig. 9).

The amplitude and duration of the contractions in the segment of the esophagus above the incision were unaltered in the majority of patients. In a few, they were decreased. The incidence of simultaneous and repetitive contractions in the segment of the esophagus not operated on also remained un-

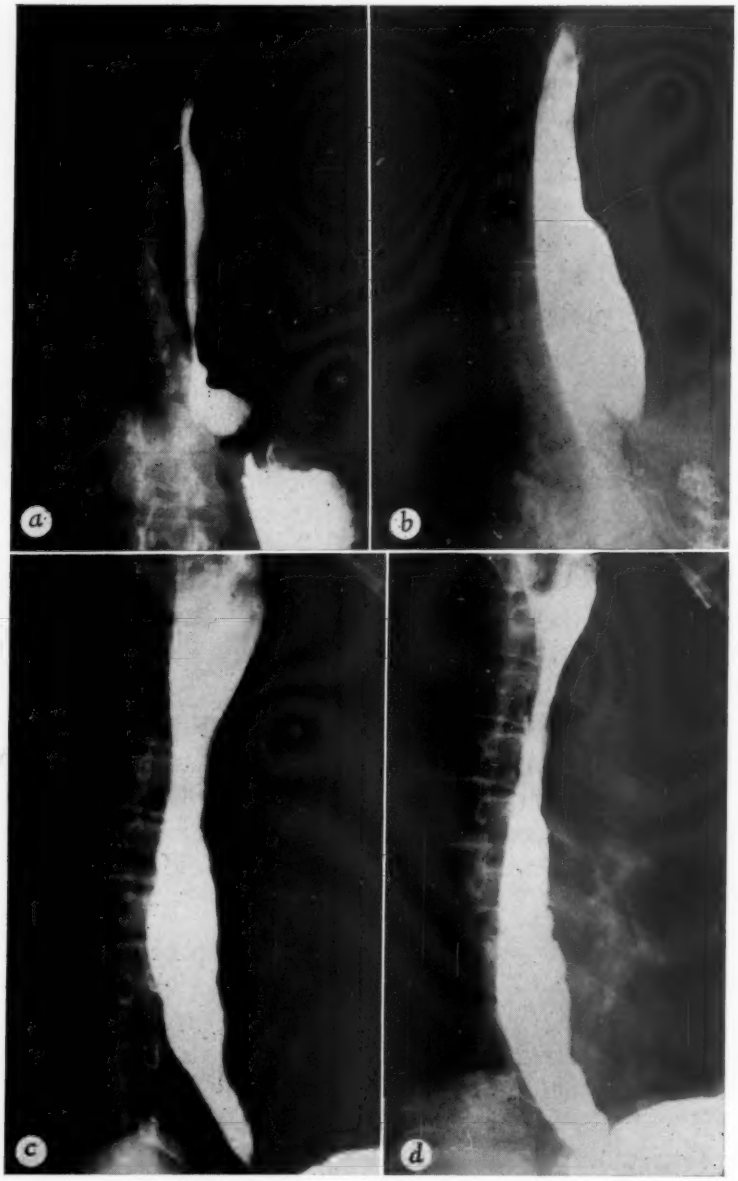


Fig. 8.—Esophageal roentgenograms in 2 patients before (*a* and *c*) and after (*b* and *d*) esophagomyotomy. Note the widening of the distal portion of the esophagus and the relief of spasm that have been accomplished by the operation.

changed. In general, little alteration occurred in the motility pattern in the undisturbed segment of the esophagus. This finding reflects the fact that, in the majority of patients, the incision purposely included all or nearly all of the affected portion of the esophagus as defined in the preoperative tests.

The gastroesophageal sphincter: The maximal pressure detected in the sphincter at rest always was reduced by the myotomy (Table III and Fig. 10). The length of the sphincter also was reduced consistently. The immediate postoperative record did not always give the clearest indication of the success of the surgical procedure. This was particularly true in patients with hypertensive gastroesophageal sphincters with diffuse spasm, in whom the lowest pressures were obtained some months after operation (Table III). In normotensive sphincters, the pressures showed little inclination to change in the

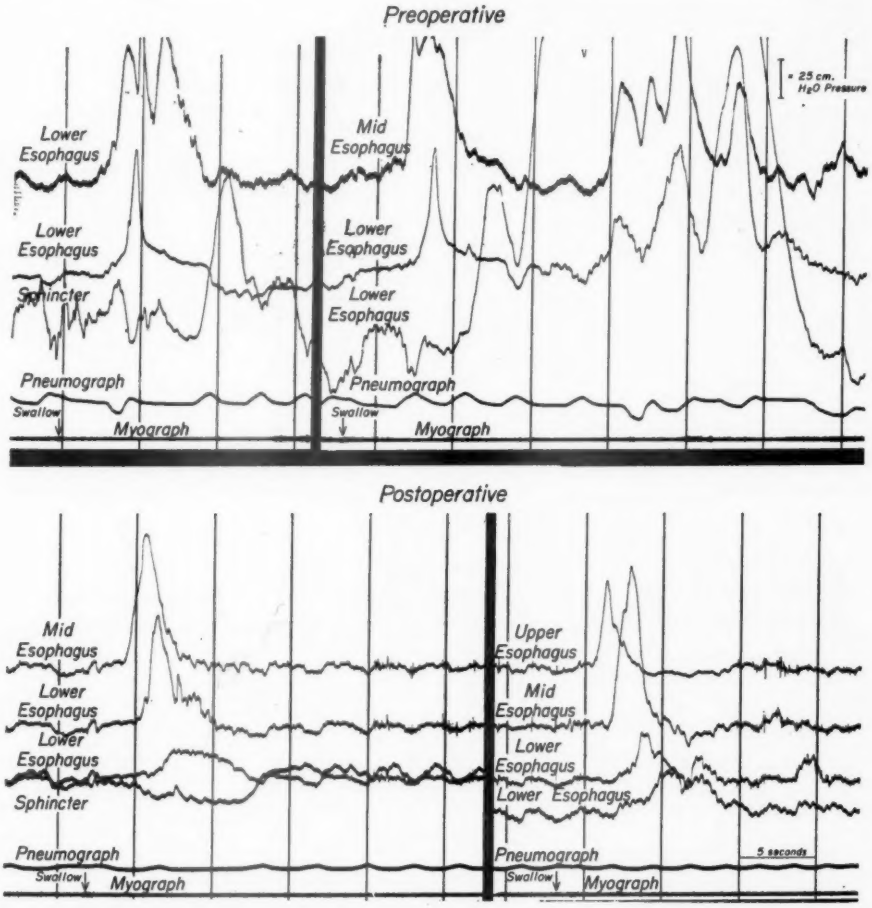


Fig. 9.—Deglutitive responses in the body and sphincter of the esophagus before *(upper 2 panels)* and after *(lower 2 panels)* esophagomyotomy. The downward-pointing arrows indicate swallows. The amplitude, duration, and repetitiveness of the contractions have been reduced by the operation in the incised region. Little change is noted in the motility pattern of the upper part of the esophagus after operation.

months following operation. Experience has indicated that relaxation and contraction of the sphincter may be interfered with or altered in the presence of a hiatal hernia. Because of the high incidence of such hernias in our patients, direct measurements of the degree of relaxation or the amplitude of contraction of the sphincter were not made.

COMMENT

Determination of esophageal motility in the study of patients with symptoms suggesting esophageal disease has disclosed a group of patients whose

TABLE III. MEAN LENGTH OF AND MAXIMAL PRESSURE AT GASTROESOPHAGEAL SPHINCTER BEFORE AND AFTER ESOPHAGOMYOTOMY*

CONDITION	PREOPERATIVE			POSTOPERATIVE								
				6-21 DAYS			3-12 MONTHS			1-3 YEARS		
	MEAN LENGTH (CM.)	MEAN MAXIMAL PRESSURE (CM. H_2O)		MEAN LENGTH (CM.)	MEAN MAXIMAL PRESSURE (CM. H_2O)		MEAN LENGTH (CM.)	MEAN MAXIMAL PRESSURE (CM. H_2O)		MEAN LENGTH (CM.)	MEAN MAXIMAL PRESSURE (CM. H_2O)	
Diffuse spasm	5.8	70	(12)	4.9	55	(10)	4.9	49	(7)	4.5	35	(6)
Diffuse spasm and hypertensive sphincter	7.6	183	(4)	5.2	115	(3)	4.6	65	(4)	—	—	
Hypertensive sphincter only	6.0	149	(2)	4.5	38	(2)	—	—		—	—	

*Numbers in parentheses indicate number of patients tested.

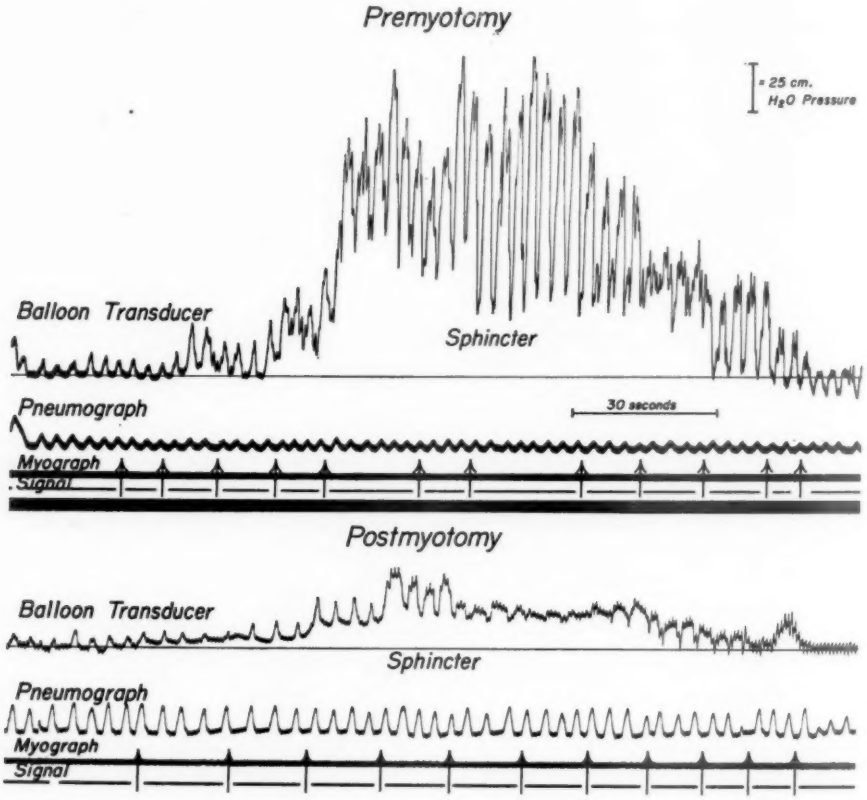


Fig. 10.—Preoperative and postoperative resting pressures at the gastroesophageal sphincter before and after esophagomyotomy in a patient with hypertensive gastroesophageal sphincter. Each upright arrow indicates a 0.5 cm. withdrawal of the recording catheter. Note the great decrease in sphincteric pressure, as well as reduction in length of the sphincter, after operation.

motility patterns were characterized by esophageal hyperactivity. This led logically to the use of an operation designed to overcome this spastic element.

Rarely do such patients have symptoms sufficiently severe to require treatment. Occasionally, however, the symptoms may be incapacitating primarily because of pain, which sometimes may be incorrectly interpreted as being of cardiac origin. Many such patients have been considered to be psychoneurotic because of the lack of objective findings. In only 4 of our 20 patients was a

diagnosis of diffuse spasm of the esophagus made by roentgenoscopy. Study of esophageal motility has supplied evidence that there may indeed be an organic basis for these patients' complaints, although the cause of the esophagospasm or the hypertensive sphincter is unknown.

The frequent finding of an associated small diaphragmatic hernia is significant. The hernia in these cases apparently is secondary to the hyperactivity exhibited by the body of the esophagus and its sphincter. Repair of the hernia alone obviously would not suffice, and this may account for some of the poor results that follow repair of a diaphragmatic hernia. Similarly, diverticula in the lower part of the esophagus may develop as a secondary phenomenon. Indeed, Effler's suggestion that all such patients should be treated by esophagomyotomy in addition to diverticulectomy has some merit. In every case, of course, it is essential that the possibility of organic esophageal obstruction be excluded. Benign and malignant tumors, strictures, and foreign bodies may result in diffuse spasm in the proximal portion of the esophagus.

The purpose of the operation that we have performed in these patients is similar to that of the procedure used for esophageal achalasia. Although normal motility cannot be restored, an attempt is made to interrupt the abnormal pattern of motility. This has been accomplished by complete longitudinal division of the esophageal musculature in the diseased zone. Preoperative studies of esophageal motility are useful in defining accurately the area to be attacked surgically.

Although the hyperactive esophageal elements are removed by operation, normal peristalsis is not restored to the operative region, and it is not surprising that all patients do not have perfect results. In fact, the number of patients whose condition has improved (84 per cent) has been less than that following esophagomyotomy for esophageal achalasia. Although one usually can predict from the postoperative esophageal studies which patients will have good results, the correlation is not always clear. Therefore, conclusions regarding the effectiveness of the operation must be somewhat guarded. The fact that so many of these patients are high-strung and nervous must be kept in mind particularly. The abnormally high incidence of incisional pain in these patients is somewhat indicative of the type of person concerned.

We have been encouraged by the early results in this small group of patients with esophageal hyperactivity treated by long esophagomyotomy. The operation should not be used indiscriminately. The operative technique should be designed meticulously to avoid the development of postoperative gastroesophageal reflux, yet the esophageal incision must be long enough to encompass the entire amount of diseased tissue. Although some patients have experienced occasional heartburn after operation, and reflux was noted roentgenoscopically in 1 of our patients, well-defined esophagitis has not developed. The initial results certainly warrant continued trial of this method.

SUMMARY

Studies of esophageal motility in the investigation of patients with esophageal disorders have led to the use of a long esophagomyotomy in the

treatment of a group of 20 patients with esophageal hyperactivity. Fourteen patients had diffuse spasm of the esophagus, 4 had diffuse spasm and a hypertensive gastroesophageal sphincter, and 2 had a hypertensive sphincter alone.

Pain and dysphagia were the predominant symptoms, with pain being the more pronounced and dysphagia occurring intermittently or not at all.

There were no deaths or complications following operation, which involved a long myotomy of the distal portion of the esophagus (8 to 15 cm. in length) and repair of an associated sliding esophageal hiatal hernia in some cases. Eighty-four per cent of the patients noted improvement after the operation.

Studies of esophageal motility carried out before and after operation not only helped to define the limits of the disease and, consequently, the area to be attacked by operation, but also provided objective evidence of the effectiveness of the operative procedure.

REFERENCES

1. Allison, P. R.: Reflux Esophagitis, Sliding Hiatal Hernia, and the Anatomy of Repair, Surg. Gynec. & Obst. **92**: 419-431, 1951.
2. Biocca, P.: Ipertrofia muscolare idiopatica dell'esofago, Arch. chir. del torace **13**: 275-304, 1956.
3. Code, C. F., Creamer, Brian, Schlegel, J. F., Olsen, A. M., Donoghue, F. E., and Andersen, H. A.: An Atlas of Esophageal Motility in Health and Disease, Springfield, 1958, Charles C Thomas, pp. 134.
4. Code, C. F., Schlegel, J. F., Olsen, A. M., and Ellis, F. H., Jr.: Hypertensive Gastroesophageal Sphincter, Proc. Staff Meet. Mayo Clin. In press.
5. Code, C. F., and Schlegel, J. F.: The Pressure Profile of the Gastroesophageal Sphincter in Man: An Improved Method of Detection, Proc. Staff Meet., Mayo Clin. **33**: 406-414, 1958.
6. Creamer, Brian, Andersen, H. A., and Code, C. F.: Esophageal Motility in Patients With Scleroderma and Related Diseases, Gastroenterologia **86**: 763-775, 1956.
7. Creamer, Brian, Donoghue, F. E., and Code, C. F.: Pattern of Esophageal Motility in Diffuse Spasm, Gastroenterology **34**: 782-796, 1958.
8. Creamer, Brian, Olsen, A. M., and Code, C. F.: The Esophageal Sphincters in Achalasia of the Cardia (Cardiospasm), Gastroenterology **33**: 293-301, 1957.
9. Effler, D. B., Barr, David, and Groves, L. K.: Epiphrenic Diverticulum of the Esophagus: Surgical Treatment, A. M. A. Arch. Surg. **79**: 459-465, 1959.
10. Ellis, F. H., Jr., Olsen, A. M., Holman, C. B., and Code, C. F.: Surgical Treatment of Cardiospasm (Achalasia of the Esophagus): Considerations of Aspects of Esophagomyotomy, J. A. M. A. **166**: 29-36, 1958.
11. van Exter, P., and Keet, A. D., Jr.: Curling of the Oesophagus, South African M. J. **28**: 206-211, 1954.
12. Heller, E.: Extramukös Cardiaplastik beim chronischen Cardiospasmus mit Dilatation des Oesophagus, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **27**: 141-149, 1913.
13. Ingelfinger, F. J.: Esophageal Motility, Physiol. Rev. **38**: 533-584, 1958.
14. Johnstone, A. S.: Oesophageal Diverticula, in Shanks, S. C., and Kerley, Peter, editors: A Textbook of X-ray Diagnosis, ed. 2, Philadelphia, 1950, W. B. Saunders Company, vol. 3, p. 36.
15. Marston, E. L., and Bradshaw, H. H.: Idiopathic Muscular Hypertrophy of the Esophagus, J. Thoracic Surg. **38**: 248-252, 1959.
16. Moersch, H. J., and Camp, J. D.: Diffuse Spasm of the Lower Part of the Esophagus, Ann. Otol., Rhin. & Laryng. **43**: 1165-1173, 1934.
17. Olsen, A. M., and Creamer, Brian: Studies of Oesophageal Motility, With Special Reference to the Differential Diagnosis of Diffuse Spasm and Achalasia (Cardiospasm), Thorax **12**: 279-289, 1957.
18. Olsen, A. M., Schlegel, J. F., Creamer, Brian, and Ellis, F. H., Jr.: Esophageal Motility in Achalasia (Cardiospasm) After Treatment, J. Thoracic Surg. **34**: 615-623, 1957.
19. Osgood, Hamilton: in Schmidt, H. W.[21]
20. Salfelder, K.: Untersuchungen über die Ösophagushypertrophie, Frankfurt. Ztschr. f. Path. **61**: 159-167, 1949.
21. Schmidt, H. W.: Diffuse Spasm of the Lower Half of the Esophagus, Am. J. Digest. Dis. **6**: 693-700, 1939.

22. Sloper, J. C.: Idiopathic Diffuse Muscular Hypertrophy of the Lower Oesophagus, Thorax 9: 136-146, 1954.
23. Sweet, R. H.: Surgical Treatment of Achalasia of Esophagus, New England J. Med. 254: 87-95, 1956.
24. Templeton, F. E.: Movements of the Esophagus in the Presence of Cardiospasm and Other Esophageal Diseases: A Roentgenologic Study of Muscular Action, Gastroenterology 10: 96-101, 1948.

Discussion

DR. PAUL NEMIR, JR. (Philadelphia, Pa.).—I think it is fitting that Dr. Ellis and his group have brought this subject before the Society. We are using the method of esophageal motility studies for a variety of lesions involving the esophagus. It has been particularly useful in diagnosis but also, in some instances, in determining the type of operative procedure that is going to be used. We have a rather unusual situation here. In the cases which Dr. Ellis presented there is a hypertonicity. In achalasia of the esophagus, while there is not necessarily a decrease in the resting tone of the esophagus, there is nonetheless a marked decrease in the amplitude of the contractions. Thus, we have one type of operation which seems to serve very well in both situations. We have had the interesting experience in several cases in which it has been extremely difficult to differentiate between a carcinoma of the lower esophagus and achalasia. The roentgen findings have not been helpful in these cases. The motility studies in the lower esophagus have indicated achalasia and in the upper esophagus have indicated some type of organic obstructive phenomena. Esophagoscopy did not add to the clarification of the problem. At operation in these cases, we have found a carcinoma of the lower esophagus and upon examination of the resected specimen, the intrinsic neural mechanism has been destroyed and replaced by tumor cells. Actually, it was almost possible to determine how high up in the wall of the esophagus the destruction of the plexus with tumor cells had occurred by changes in the motility at various levels of the esophagus.

I would just like to ask Dr. Ellis if biopsies of the muscle have been taken at the time of operation and if he can give us any idea of what the plexus of Auerbach was like in these studies. In most of our cases of achalasia of the esophagus we have not found an absence of the Auerbach plexus but rather a decrease in the number of ganglion cells. It would seem that the altered motility is on the basis of a derangement of function of the intrinsic neural mechanism rather than an absence of it.

DR. F. HENRY ELLIS, JR. (closing).—I would like to thank Dr. Nemir for his remarks. We have only performed a few biopsies of the distal esophageal muscle and ganglion cells were present in all. Biopsies of this sort probably do not give a fair sample of what is present in the wall of the distal esophagus.

I think it is extremely important to exclude organic diseases as a cause of pain or dysphagia and Dr. Nemir's remarks regarding the presence of malignant tumors is very apropos, I think. Patients can have diffuse spasm of the esophagus, secondary to any obstruction, and the physiologist cannot differentiate between the various possible causes. The clinician is the one who has to decide whether the patient has a malignant tumor as the cause of his disordered motility or not.

Another question that perhaps should be mentioned is the long-term follow-up. Do these patients maintain their immediate postoperative motility pattern, or is there a change later on toward normal or toward the original diffuse spasm picture? In the patients whom we have studied, up to 3 years after operation, the resting pressures in the operative area have not changed. There has neither been a return of the preoperative abnormal pattern nor has there been a return of peristalsis. I think perhaps one reason for this is that our operative technique consists not only of making an incision in the distal musculature, but also, in a sense, actually decorticating the mucosa of the lower esophagus so that there will be no opportunity for the muscle edges to agglutinate and re-form a circular muscular ring around the esophagus.

INTRACTABLE PEPTIC ULCER AND ENDOCRINE ADENOMAS WITH PITUITARY AMPHOPHILIC HYPERPLASIA

A Reinterpretation of the Ellison-Zollinger Syndrome

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ZOLLINGER and Ellison[50] have described a syndrome consisting of progressive and intractable peptic ulceration (often atypical in location) with non-insulin-producing islet cell tumors of the pancreas and gastric hyperacidity. The identification and separation of this interesting group of patients from the large group of those suffering from duodenal ulcer has been an important step forward in the understanding of this common disease. It may call attention to endocrine mechanisms underlying a much larger fraction of instances of the disease when it is less spectacular in its manifestations.

Up to this time approximately 81 cases have been recorded that appear to fit into this general category. Of the patients recorded in the literature, about 25 per cent have been shown to have other endocrine adenomas in addition to the pancreatic pathologic findings. Also, Ellison,[15] in a study of postmortem material at the Ohio State University Hospital, has shown an increased incidence of duodenal ulcer in diabetes mellitus, and a significant correlation between duodenal ulcer and the occurrence of adrenal cortical adenomas unaccompanied by any signs of hypercorticism.

A syndrome of this type was first described by Sauler and Zinninger[42] in 1946. The case was that of a 40-year-old woman with a bleeding duodenal ulcer. There was no clinical or biochemical evidence of hyperinsulinism and at operation an islet cell carcinoma of the pancreas was removed. At that time metastases were noted in the liver. The patient died as a result of advancing malignancy.

It is the purpose of this paper to describe the case of a patient suffering from this complex disorder and to present data from this case and from the literature suggesting that the syndrome may be of pituitary origin.

CASE REPORT

Clinical Data.—A 57-year-old salesman, M. A., gave a history of a duodenal ulcer with intermittent activity for several years and a major hemorrhage 4 years previously. For the several months prior to admission midepigastric pain associated with postprandial vomiting was a prominent feature. The patient had noted occasional black stools but was not troubled with diarrhea.

Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

This work was done under an American Cancer Society Clinical Fellowship.

The patient was hospitalized in March, 1956, for obstruction and bleeding for a period of 30 days. Treated by conservative measures, he regained a condition that was considered satisfactory for discharge despite continuing borderline obstruction. He had received 1,500 ml. of whole blood.

Following discharge clinical evidence of obstruction again became much more prominent with abdominal pain, emesis, and upper abdominal swelling.

On his second admission in May, 1956, physical examination showed a man in considerable distress, hiccoughing and belching.

Blood pressure was 160/90 mm. Hg and pulse 74. Initially there was abdominal swelling relieved by aspiration of 1,500 ml. of guaiac negative, highly acid contents. X-rays confirmed the obvious gastric obstruction.

The initial laboratory work revealed urine specific gravity 1.023, pH 7.5, and stool guaiac test negative. Hematocrit was 45 per cent, white blood cells 16,500 per cubic millimeter, Na 140 mEq. per liter, K 2.5 mEq. per liter, and CO_2 combining power 35.4 mEq. per liter. Gastric drainage for the first 24 hours totaled 4,000 ml. and a cumulative 3-day total was 12,700 ml. The gastric juice electrolyte determinations revealed Cl 150 mEq. per liter, K 10 to 15 mEq. per liter, urine K 104 mEq. per liter and Na 115 mEq. per liter. The patient was treated for the hypochloremic, hypokalemic alkalosis and 2 days later the following blood data were reported: pH 7.55, CO_2 tension 47 mm. Hg, total CO_2 41 mM. per liter, CO_2 combining power 46.3 mM. per liter, Cl 73 mEq. per liter, Na 138 mEq. per liter, and K 2.0 mEq. per liter.

More vigorous treatment of the severe alkalosis was undertaken and on May 14, 1956, the patient underwent a first stage gastrectomy. At operation the only abnormality noted was marked edema of the duodenum with multiple adhesions to surrounding structures. Postoperatively, the patient did well. The patient was discharged and readmitted, and underwent the second stage of the gastrectomy on June 25, 1956.

Postoperatively, he continued to progress well and was followed in the Outpatient Department where he complained of inability to swallow solid foods often associated with vomiting. Over the next few months he began to lose weight, was noted to have occult blood in the stools and a hypochromic, microcytic anemia. Two gastrointestinal x-rays were unrevealing and the anemia responded to parenteral iron therapy.

His complaints continued and he was again admitted to the hospital on Nov. 21, 1956. Initial laboratory work revealed hematocrit 42 per cent, urine negative, white blood cells 10,000 cells per cubic millimeter, BUN 7, Na 136, K 3.7, CO_2 16.2, and Cl 99. The stool was positive for blood. The urine uropepsin value was 9,763 units per 24 hours. X-rays revealed marked pylorospasm, with narrowing at the esophagogastric junction. Sigmoidoscopy was negative. Gastric aspiration showed benzidine negative material without free acid. Esophagoscopy showed acute inflammation and narrowing in the lower one third of the esophagus. Biopsies were taken which showed chronic inflammation. Attempts at bouginage were unsuccessful.

On the patient's fifteenth hospital day he complained of sudden epigastric pain radiating to the right shoulder. He was found to be hypotensive and was given blood, plasma, vasopressors, and antibiotics. There was evidence of rapidly progressing peritonitis. The patient died while being prepared for operation 12 hours after the onset of the acute episode.

Gross Findings.—A postmortem examination was performed. The peritoneal cavity contained 1,650 ml. of sanguineous, seropurulent fluid and the serosa was covered in part by a thin layer of purulent exudate. A perforation of the jejunum (2 cm. diameter) was found in the anterior wall 1.5 cm. distal to the gastrojejunal anastomosis. Two centimeters distal to the perforation was an acute ulcer (1 cm. diameter) which had penetrated into the muscularis (Figs. 1 and 2). Several centimeters distally there was a small group of acute ulcers.

The gastrojejunal anastomosis was intact and well healed with the gastric portion measuring 9.5 cm. from the esophagogastric junction to the anastomosis. The gastric mucosa was hyperplastic and had scattered petechiae.

Fig. 1.

Fig. 2.

Fig. 1.—Esophagus, gastric segment, and jejunum. The upper arrow indicates the ulceration of the hypertrophic esophagus. The lower arrow points to the jejunal perforation as seen from the serosal surface.

Fig. 2.—Gastrojejunal anastomosis with jejunal ulceration and perforation. The white strip indicates the site of perforation. The arrow points to one of the ulcers in the jejunum.

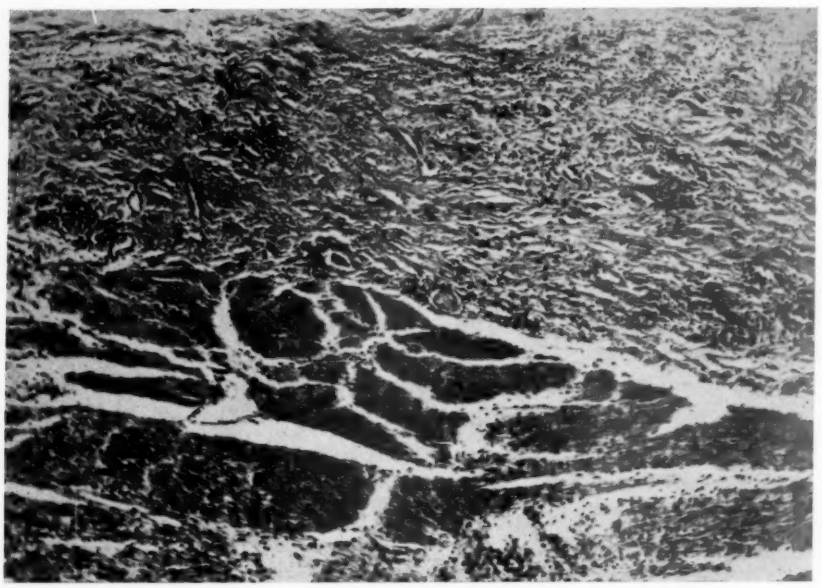

Fig. 3.—Esophagus. The acute and chronic inflammatory process has resulted in loss of mucosa, and cellular infiltration, and fibrosis of the submucosa with extension into the muscularis. (Hematoxylin and eosin; original magnification ×85.)

The esophagus was narrowed at its entrance into the stomach. The mucosa here was normal but the muscularis was hypertrophied. Proximally (Fig. 3) the mucosa was eroded and hyperemic with the muscularis visible. The wall here also was thickened and the circumference measured 4 cm. The distal 8 cm. of the esophagus was rigid and tubelike. The more proximal portion had a normal mucosa and circumference of 8 cm.

In the duodenum there was a healed ulcer with fibrosis extending into the submucosa and muscularis. One centimeter distal to the ampulla of Vater there was a firm 5 mm. nodule in the wall of the duodenum.

At the junction of the body and tail of the pancreas there was a 6 mm. firm, gray, rounded mass which proved microscopically to be an islet cell adenoma not containing beta cells. In the right adrenal gland was an adenoma which measured 4 by 3 by 2 cm. The right adrenal gland weighed 32 Gm. and the left 20 Gm. The pituitary was normal in size.

There was bilateral bronchopneumonia with a small abscess (8 mm. in diameter) in the right upper lobe. The marrow was hyperplastic. The thyroid was somewhat small (19 Gm.) but otherwise normal grossly. The heart weighed 370 Gm., and had slight atherosclerosis of the coronary arteries. The liver was normal in appearance except for moderate centrolobular congestion and relatively small size (1,200 Gm.).

Microscopic Findings.—The ulcerative process in the jejunum was acute with no evidence of repair and with abundant necrotic mucosa. There was little cellular reaction even adjacent to the side of perforation (Fig. 4). In the esophagus (Fig. 3) the ulcerative process was active and chronic with fibrosis and cellular infiltration of the muscularis.

There was no abnormality of the acinar tissue of the pancreas, save for some foci of replacement by adipose tissue. Occasional islets of Langerhans were enlarged and had a hyperplastic cordlike arrangement of the cells (Fig. 5). The adenoma (Fig. 6) consisted of cells in which no beta granules could be demonstrated by the Gomori chrome alum hematoxylin-floxine method. Since no tissue had been fixed in a nonaqueous media, it was not possible to stain for alpha granules.

In the adrenal cortex (Fig. 7) there were rounded foci suggestive of cortical hyperplasia and containing little lipid. The deeper portions of the fasciculas had abundant, granular, brownish pigment. The adenoma of the cortex consisted of sheets of vacuolated cells and was well demarcated.

In the pituitary (Fig. 8) there was an abundance of basophilic cells, sparsely granulated periodic acid Schiff positive cells and amphophil cells, occasionally in an acinar arrangement. In some areas acidophils were numerous, but there was a general reduction in the number of chromophobe cells. In the stomach (Fig. 9), which consisted of cardiac and proximal fundic portions only, the mucosa was thick and contained a marked overabundance of parietal cells. No ulceration was noted.

In the duodenum, the nodule adjacent to the ampulla of Vater consisted of cells in both cordlike and acinar arrangements, and was identified as a hamartoma. The parathyroids were normal with reference to cell type and amount of adipose tissue. There were no adenomas and there was no evidence of hyperplasia.

The bone marrow was moderately immature in the granulocytic series and there was slight hyperplasia of the erythrocytopoietic series. In the lungs, there were small foci of bronchopneumonia and in a section from the right upper lobe there was an abscess in the center of an area of bronchopneumonia. In the kidneys, there was focal atrophy related to arteriosclerosis. There was advanced centrolobular congestion in the liver with moderate atrophy of liver cells. There were no abnormalities noted microscopically in the heart, the spleen, the prostate or the thyroid. The gonads were not examined.

Discussion.—The patient presented a number of interesting problems. His case may be summarized as embodying: duodenal ulcer, jejunal ulcers with perforation, esophagitis with stricture, gastric parietal cell hypertrophy, gastric hypersecretion, duodenal hamartoma, pancreatic islet-cell hyperplasia and adenoma, adrenal hyperplasia and adenoma, and pituitary amphophilic hyperplasia.

His extreme gastric hyperacidity was first manifested by the severe hypochloremic, hypokalemic alkalosis when obstructed. Of note also is the high gastric antral output

Fig. 4.

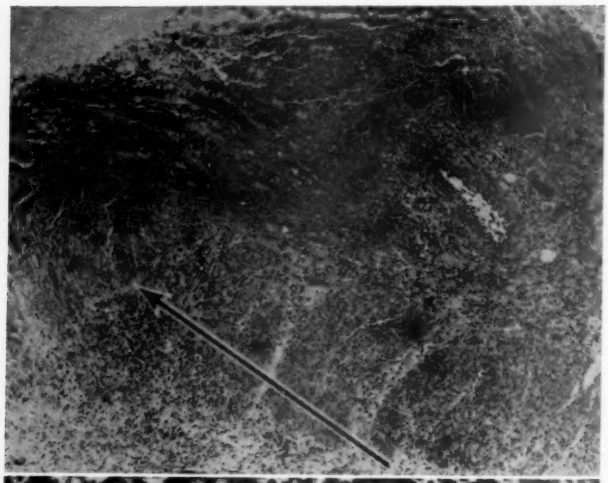

Fig. 5.

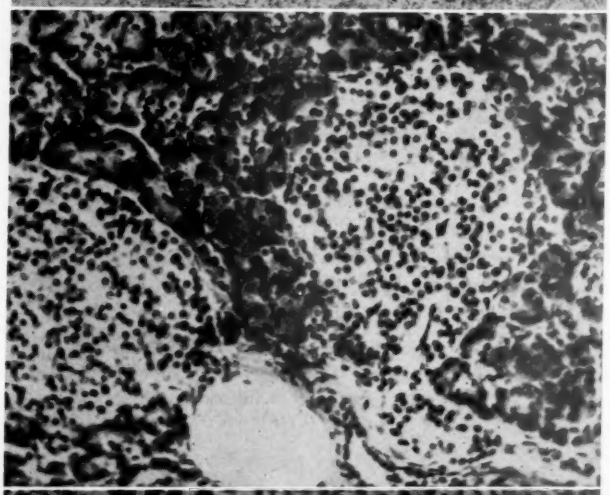

Fig. 6.

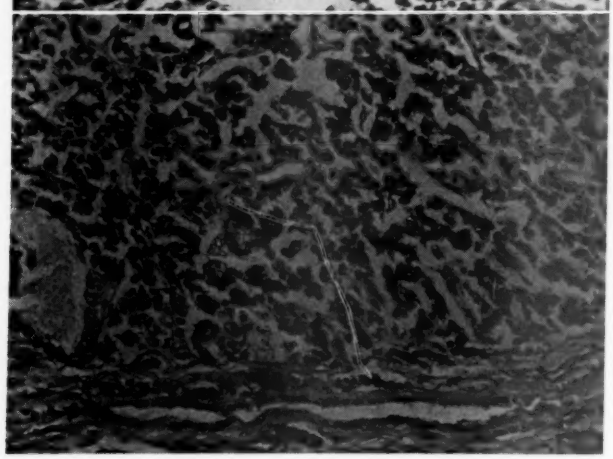

Fig. 4.—Jejunum: Acute peptic ulcer. The acute ulcerative process has destroyed the mucosa and in the right half of the field extends into the submucosa, in the left half *(arrow)* has produced necrosis of the muscularis propria. (Hematoxylin and eosin; original magnification ×85.)

Fig. 5.—Pancreas: Islets. There is a cordlike arrangement of the cells in portions of these enlarged hyperplastic islets. (Hematoxylin and eosin; original magnification ×260.)

Fig. 6.—Pancreas: Adenoma of islet cells. The cells are uniform in size and no beta granules were demonstrable with a specific stain for granules. A portion of a thin, fibrous capsule is present at the bottom of the field. (Hematoxylin and eosin; original magnification ×230.)

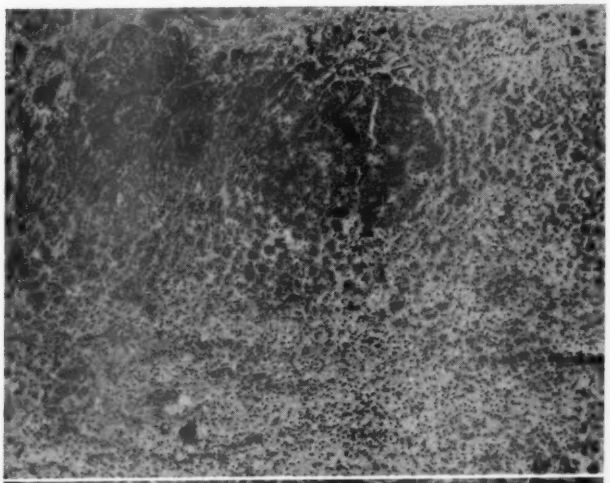

Fig. 7.

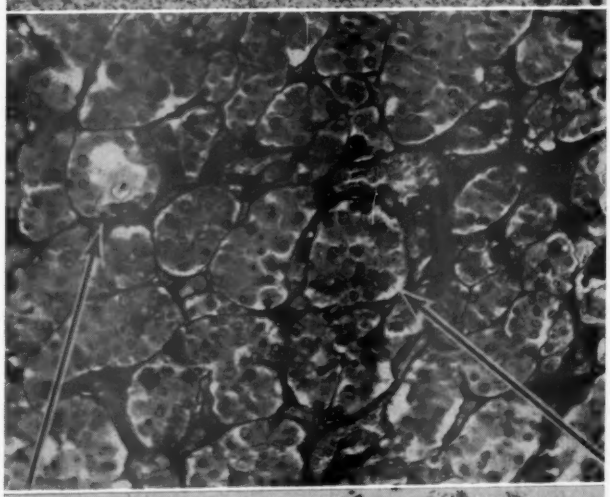

Fig. 8.

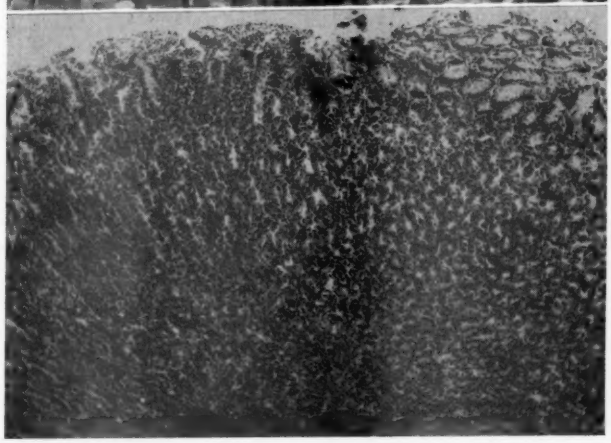

Fig. 9.

Fig. 7.—Adrenal cortex. In this hyperplastic gland, there are 2 nodules with extension into the capsule in the upper portion of the field. (Hematoxylin and eosin; original magnification ×85.)

Fig. 8.—Hypophysis: Anterior lobe. The predominant cell in this hyperplastic gland is enlarged and is sparsely granulated or nongranular. Acinus-like formations are present with basal position of the nuclei. Two such formations are indicated by arrows. (Periodic acid Schiff orange G; original magnification ×260.)

Fig. 9.—Stomach. The mucosa is thick and there is an abnormal abundance of parietal and chief cells, especially the former, for this proximal portion of the stomach. (Hematoxylin and eosin; original magnification ×70.)

between the stages of the gastrectomy. These facts emphasize that some potent stimulus to gastric secretion presumably was present.

The overabundance of parietal cells in the fundus is unusual in that there should be approximately one half as many parietal cells in the fundus as there are in the body of the stomach. The fundic glands should contain more mucous and should have more branching glands of the type found at the cardia and also near the pylorus. If one estimates the total area of the stomach by an addition of those portions removed to what remained, the total area is somewhat over 400 cm.^{2}. This is not an accurate figure but a fair estimation. According to the figures of Cox[4] an area of 400 cm.^{2} places this stomach into the smaller size group. The average size for a normal stomach is about 850 cm.^{2}. Those patients with duodenal ulcers usually have stomach areas larger than 850 cm.^{2} and in those with gastric ulcers the area is smaller than this figure. Again, according to Cox, the number of parietal cells is larger in the stomach of a patient with a duodenal ulcer than in a patient with a gastric ulcer or a normal stomach. The number of parietal cells in normal stomachs averaged 0.8 billion and in cases of duodenal ulcer the average is 1.8 billion. Interestingly, in our case there was a stomach that was much smaller than the average but contained an overabundance of parietal cells in a portion of the stomach that does not usually have the preponderance of acid-producing cells.

The time interval between the second stage of the gastrectomy and the jejunal perforation was short (5 months). In fact, the resection itself might be considered to have accelerated the basic disease process. Removal of the remaining gastric tissue may have released other humoral factors from a previously inhibiting influence.

This case is not unlike that of other reported patients with the Ellison-Zollinger syndrome. This combination has been characterized by gastric hypersecretion and the presence of primary peptic ulceration in unusual locations, that is, second or third portions of the duodenum, the jejunum, or recurrent marginal ulcerations in patients previously subjected to various types of gastric operation. The usual pathologic findings, either at the time of re-exploration or autopsy, have shown pancreatic adenomas, usually of the islet cell type. Most of these have not been insulin-producing and have been of the alpha cell variety.

The typical patient has been a woman in the 40 to 60 year age group with ulcer symptoms of varying duration who has been subjected to a number of surgical procedures aimed at obliterating the source of acid production or its mediation. The usual postoperative course has been one of recurrent ulcer pain with continued gastric hypersecretion except in a few cases in which a partial or total pancreatectomy has been carried out. In this latter group of patients only a few have been treated by the removal of the pancreas and/or its adenomas and this has not consistently been effective. There has been no definite etiological evidence as to why such pancreatic adenomas should be a causative factor in gastrointestinal ulceration. Some investigators have mentioned glucagon[14] in relation to ulceration; however, experimental evidence[11] is not conclusive and at present even though this may be produced by pancreatic adenomas we cannot say this is a definite causative agent.

Of particular interest here was the finding of pituitary pathology of a type often found where pituitary stimulation of target endocrine organs is very active.

DISCUSSION

Our interest in this syndrome has centered about the occurrence of peptic ulceration in combination with endocrine adenomas and the possible pituitary etiology of both. In the brief review that follows, evidence bearing on this point will be gathered under 4 headings, leading to a hypothesis as to the pathogenesis of this syndrome.

Endocrine Aspects of Duodenal Ulcer.—It has been known for a long time that many of the endocrinopathies are associated with an increased or decreased incidence of acid-peptic disease. We have described below some of the pertinent information concerning endocrine hormones and peptic ulceration.

Gonads: The incidence of duodenal ulcers is much higher in men than in women. The usual ratio given is 3 to 1. It has also been observed that ulcer symptoms frequently undergo marked changes with change in the steroid environment during pregnancy and with the onset of the menopause.

Experimental work with Mann-Williamson dogs has shown that these preparations develop fewer ulcers when given estrogens or anterior-pituitary-like hormones.[3] These same results hold true for animals with gastroileostomies.

Parathyroids: The relationship between primary hyperparathyroidism and peptic ulceration has been stressed in the literature.[1, 20, 25, 34, 43] B. M. Black, in Haynes' monograph,[20] states that 24 per cent of patients with hyperparathyroidism have peptic ulcers and another 15 to 20 per cent have gastrointestinal symptoms although an ulcer is never conclusively demonstrated.

In an article by Ostrow, Blanshard, and Gray,[36] a discussion of 39 patients from this hospital with primary hyperparathyroidism, the incidence of proved peptic ulceration was 7.7 per cent. Two of the patients with peptic ulcers also had multiple endocrine tumors and pancreatic tumors. One additional patient had multiple endocrine adenomas without peptic ulceration. They compiled the results from all of these cases reported in the recent literature and noted a 9.1 per cent occurrence of peptic ulcer with hyperparathyroidism. They considered the distinctive features of ulceration with hyperparathyroidism to be (1) the greater number of duodenal ulcers in women (6.2 per cent of 288 patients had ulcers in contrast with a loosely estimated sex-age-corrected populational incidence of 1 to 100,000), (2) the increased frequency of gastric ulcers in men, and (3) the frequent absence of gastric hypersecretion.

Thyroid: The incidence of peptic ulceration appears to be lower in persons with thyrotoxicosis than in normal individuals. It has been noted that the volume and acidity of gastric juice is decreased and Louis and Wills[27] pointed out that thyrotoxicosis is one of the important causes of hypo- or achlorhydria. In their series of 18 patients, 8 had achlorhydria and 7 had hypochlorhydria. Eight to 10 days after thyroidectomy, 12 out of 15 showed a return to normal or an increase in the free acid.

Pancreas: In their book on peptic ulcer, Ivy, Grossman, and Bachrach[23] mention the fact that patients with diabetes mellitus are not more susceptible to peptic ulcers than normal individuals. They quote Joslin's[24] experience of not having seen an ulcer in the first 2,700 cases of diabetes. Wilder[47] noted a 2.3 per cent incidence of ulcers in diabetes while during the same period of time the incidence of peptic ulceration at the Mayo Clinic was 3.5 per cent. Wood and Rothenberg[38, 49] reported an ulcer incidence of 0.78 per cent and 0.25 per cent, respectively, in a total of 15,525 diabetic patients. Ellison, Abrams, and Smith[15] contradict most of the previous literature on this subject and in their review of 812 gastrointestinal ulcers in 20,000 consecutive autopsies they noted 37 of the ulcer patients with diabetes mellitus, an incidence of 4.6 per cent, whereas the incidence of diabetes in the general population is 1.5 per cent.

Adrenals, Antrum, and Pituitary.—Patients with untreated Addison's disease are known to have a very low incidence of peptic ulceration and in a series

of 363 cases, Gray[19] reports only 3 persons with a chronic peptic ulcer. It has also been shown[39] that about 50 per cent of patients with Addison's disease have achlorhydria, decreased levels of chloride in the gastric juices, and low levels of blood pepsinogen and urinary uropepsins. Administration of adrenocorticotrophic hormone and cortisone returns all these values to their normal levels. In animals which have undergone bilateral adrenalectomy, there is no gastric response to the administration of adrenocorticotrophic hormone, while in normal animals[46] or individuals adrenocorticotrophic hormone or cortisone stimulates gastric acid and pepsin secretion. This stimulation is via a hormonal pathway as has been shown in vagally denervated, antrectomized preparations. In patients who have been studied following bilateral adrenalectomy there is a decrease in both gastric acidity and pepsin secretion. Of note also is the finding that the incidence of peptic ulcer is higher in patients with Addison's disease treated with corticoids than in those requiring no treatment.

Patients with Cushing's disease are of interest because they, as well as those with the adrenogenital syndrome, rarely are found to have an ulcer of any type.

In patients with prolonged "stress," such as thermal burns, there is an increased incidence of ulcers and gastritis if they survive the initial 2 to 3 weeks.[22] In Moore's[31] article it is pointed out that the adrenal corticoid changes in burns are of a peculiarly long duration and are associated with gross and microscopic adrenal hypertrophy.

Ellison[15] showed that 13.2 per cent of 812 patients with gastroduodenal ulceration demonstrated hyperplastic lesions of the adrenals.

Edkins[12] noted that when various portions of the stomach were ground up and given intravenously only those taken from the antral region increased gastric secretion. Atropine did not decrease the secretory response. McKittrick, Moore, and Warren[28] re-emphasized the importance of the antral mucosa in their description of the two-stage gastrectomy and the necessity of antrectomy in exclusion procedures. They showed that most patients developed jejunal ulcers when the antrum was left after a Finsterer-type procedure. Since then, much work has been done to establish the endocrine function of the antrum.[8-10]

The antral mucosa occupies an impórtant position in secretory activity of the stomach and at the present time we can classify it as a true endocrine organ, though we know little of its endocrine relations.

There is also evidence to show that in experimental animals hypophysectomy is followed by involution of the gastric chief cells and depletion of pepsinogen granules.[2] These effects are probably not caused by the lack of corticoids or thyroid hormone and are reversed by the administration of somatotrophic hormone.

The Syndrome of Multiple Endocrine Adenoma.—Many authors have described patients burdened by multiple endocrine adenomas. Some of these tumors have been functioning while others produced no obvious clinical activity. Lloyd,[26] in 1929, was probably the first to describe such a syndrome. His patient was a 22-year-old girl with an inoperable chromophobe adenoma in association with adenomas of the parathyroids and islets of Langerhans. Ström[44] described a 32-year-old patient with ulcers with what was probably a nonfunctioning

delta cell tumor of the pancreas. Subsequently, Moldawer, Nardi, and Raker[30] described a syndrome of multiple endocrine tumors with a familial incidence. Twenty-five per cent of these patients had peptic ulcers. They concluded that the occurrence of multiple endocrine tumors was a genetic phenomenon.

Russfield, Renier, and Klaus[40] reported on 27 patients with multiple endocrine tumors, 3 of whom had peptic ulcers. In all of their patients they showed some histologic evidence of "endocrine target–organ failure" and what they presumed to be pituitary amphophilic hyperplasia secondary to this end-organ failure and associated with a high level of pituitary trophic activity. Underdahl, Woolner, and Black[45] described 8 patients with endocrine adenomas in 2 or more glands. Three of this group had peptic ulcers and 1 had diffuse gastric polyposis. Of the 3 patients with peptic ulcers proved at operation, 2 had pituitary adenomas at the time of autopsy.

Pituitary Relations in Endocrine Adenomas.—In many of the cases of multiple endocrine tumors that have been reported,[42, 44] no mention is made of pituitary pathology but in others[13, 15, 26, 30, 40, 45] it is striking to note the associated pituitary adenomas and/or hyperplasia. Conversely, Putnam[37] points out the striking incidence of target-organ adenomas or hyperplasia in a primary pituitary dysfunction such as acromegaly. In Ellison's[15] 24 cases, out of a group of 5 patients who had tumors of other endocrine organs as well as the pancreas, 2 had acromegaly. Russfield and associates[40] noted 3 cases of proved peptic ulcer in 28 individuals with hypophyseal tumors. One of the 3 had acromegaly. Cushing's[5] description of basophil pituitary adenomas included a number of patients who showed hyperplasia or adenomas of endocrine glands other than the adrenals.

Concept of End-Organ Failure.—Russfield,[41] in Mellor's text, has developed some interesting concepts regarding the pattern of pituitary changes associated with end-organ hypoactivity of 1 gland and overactivity of the others.

In both man and experimental animals, pituitary amphophilic hyperplasia has been noted following total body irradiation, pregnancy, the administration of exogenous target-organ hormone from a single gland, and target-organ deficiency of a single gland. Russfield has also pointed out that the amphophil cells, as stained by the periodic acid Schiff orange G technique, are the source of some of the trophic hormones. Once activated by target-organ deficiency, these cells increase in number and can produce one or all of the trophic hormones in increased amounts. It is this elevated trophic hormone level that is responsible, in the opinion of Russfield, for the subsequent development of hyperplasia or adenomas in one or more of the endocrine glands.

Studies on laboratory induction of endocrine tumors lend further credence to this "feed-back" concept. Furth[16-18] has been able to produce thyrotropic, adrenotropic, and mammotropic hormone-secreting transplantable pituitary tumors in mice by inducing a marked and sustained depression in the level of the target-organ hormone in irradiated animals.

Dickie and Woolley[6] have shown that gonadectomized mice will show extensive alveolar development of the mammary glands, adrenal cortical tumors,

and basophilic tumors of the pituitary. The mammary and adrenal gland changes always proceeded the changes in the pituitary.

McManus and Sommers[29] note that 70 per cent of women with carcinoma of the breast show ovarian cortical stromal hyperplasia. This may be interpreted as related to increased gonadotrophin levels secondary to mammary gland failure. Wilson and his group[48] cite a few patients with carcinoma of the breast whose disease was accelerated by oophorectomy. In these individuals mammotrophin output may have been increased by ovarian and organ ablation. Morse, Criscitiello, and colleagues[33] report a case of a 34-year-old man who underwent a bilateral adrenalectomy for hypertensive vascular disease. At the time of postmortem examination 1 year later there was pituitary amphophilic hyperplasia, increased amounts of melanin in the basal layer of the epidermis, and focal hyperplasia of the acinar tissue and of the islands of Langerhans in the pancreas.

Nelson and co-workers[35] described a patient with hyperadrenocorticism who underwent a bilateral adrenalectomy and 3 years later was found to have a large chromophobe adenoma of the pituitary gland that secreted large amounts of adrenocorticotrophic hormone. This adrenocorticotrophic hormone production could not be suppressed, as in patients with adrenal insufficiency, by the administration of intravenous hydrocortisone. There was also evidence for elevated amounts of follicle-stimulating and melanocyte-stimulating hormones in the circulating blood.

Thus there is a mounting body of evidence, both experimental and clinical, to support the concept that endocrine-organ "failure" or hypofunction can bring about hyperplastic and adenomatous changes in the pituitary, manifested either in the amphophilic or basophilic cells.

With these facts in mind one may advance a hypothesis that would explain the pathology found in 1 patient and in an unknown additional number of patients suffering from endocrine adenomas and duodenal ulcer.

Hypothesis.—Such a hypothesis holds that there has been a relative or absolute failure of secretion of one of the target-endocrine organs. This has in turn brought about hyperplastic and, in some cases, adenomatous changes in amphophil or basophil cells of the pituitary gland. These changes are reflected in an increased production of one or more of the trophic hormones. These in turn cause hyperplasia or adenomas of the other target-endocrine organs. The acid-peptic axis is stimulated directly through antral activity and parietal cell hyperplasia or indirectly as a by-product of secretion of the adrenals, gonads, or parathyroids. Intractable ulcer results.

SUMMARY

1. The syndrome of intractable peptic ulceration and multiple endocrine tumors is discussed.

2. The influence of the endocrine glands on the acid-peptic mechanism has been reviewed along with some of the pituitary relations in multiple endocrine tumors.

3. Evidence is brought forward to suggest that the pituitary gland is the prime mover in cases associated with endocrine tumors, and that a possible pituitary activating mechanism is end-organ failure.

4. A case has been presented that is representative of the Zollinger-Ellison syndrome but also involves adrenal hyperplasia and adenoma, parietal cell hyperplasia and amphophilic hyperplasia of the pituitary. No "initiating" end-organ failure was identified in this case.

REFERENCES

1. Albright, H. L., and Kerr, R. C.: Primary Hyperplasia of Parathyroid Glands: Report of a Case With Coincidental Duodenal Ulcer, J. A. M. A. **148**: 1218, 1952.
2. Baker, B., and Abrams, G.: Hypophyseal Growth Hormone, Nature and Actions, New York, 1955, McGraw-Hill Book Company, Inc.
3. Broad, G. G., and Berman, L. G.: Treatment of Experimental Mann-Williamson Ulcers With Anterior-Pituitary-like Hormone, Am. J. Digest. Dis. **8**: 27, 1941.
4. Cox, A.: Stomach Size and Its Relation to Chronic Peptic Ulcer, A. M. A. Arch. Path. **54**: 407, 1952.
5. Cushing, H.: Basophilic Adenomas of the Pituitary Body, Bull. Johns Hopkins Hosp. **50**: 137, 1932.
6. Dickie, M., and Woolley, G.: Spontaneous Basophilic Tumors of Pituitary Glands in Gonadectomized Mice, Cancer Res. **9**: 372, 1949.
7. Donaldson, R., von Eigen, P., and Dwight, R.: Gastric Hypersecretion, Peptic Ulceration and Islet-Cell Tumor of the Pancreas, New England J. Med. **257**: 965, 1957.
8. Dragstedt, L. R.: Physiology of Gastric Antrum, A. M. A. Arch. Surg. **75**: 552, 1957.
9. Dragstedt, L. R., Oberhelman, H. A., Jr., Evans, S. O., and Rigler, S. P.: Antrum Hyperfunction and Gastric Ulcer, Ann. Surg. **140**: 396, 1954.
10. Dragstedt, L. R., Oberhelman, H. A., Jr., and Smith, C. A.: Experimental Hyperfunction of Gastric Antrum With Ulcer Formation, Ann. Surg. **134**: 332, 1951.
11. Earle, A. S., Cahill, G., and Hoar, C.: Studies on the Relationship of Glucagon (HGF) to Gastric Function, Ann. Surg. **146**: 124, 1957.
12. Edkins, J.: The Chemical Mechanism of Gastric Secretion, J. Physiol. **34**: 133, 1906.
13. Eiseman, B., and Maynard, R.: Non-Insulin Producing Islet-Cell Adenoma Associated With Progressive Peptic Ulceration, Gastroenterology **31**: 296, 1956.
14. Ellison, E.: Ulcerogenic Tumors of the Pancreas, SURGERY **40**: 147, 1956.
15. Ellison, E., Abrams, J., and Smith, D.: A Postmortem Analysis of 812 Gastroduodenal Ulcers Found in 20,000 Consecutive Autopsies With Emphasis on Associated Endocrine Disease, Am. J. Surg. **97**: 17, 1959.
16. Furth, J.: Conditioned and Autonomous Neoplasm. A Review, Cancer Res. **13**: 477, 1953.
17. Furth, J.: Discussion of Problems Related to Hormonal Factors in Initiating and Maintaining Tumor Growth, Cancer Res. **17**: 454, 1957.
18. Furth, J.: Experimental Pituitary Tumors, Recent Progr. Hormone Res. **11**: 221, 1955.
19. Gray, S. J.: Relationship of the Adrenal Gland to Peptic Ulcer, M. Clin. North America **41**: 1471, 1957.
20. Haynes, A. L., in Black, B. M.: Hyperparathyroidism, Springfield, 1953, Charles C Thomas, Publisher.
21. Hellstrom, J.: Hyperparathyroidism and Gastro-Duodenal Ulcer, Acta chir. scandinav. **116**: 207, 1958-59.
22. Hummel, R. P., Lanchantin, C. F., and Artz, C. P.: Clinical Experiences and Studies in Curling's Ulcer, J. A. M. A. **164**: 141, 1957.
23. Ivy, A. C., Grossman, M. I., and Bachrach, W.: Peptic Ulcer, Philadelphia, 1950, The Blakiston Company.
24. Joslin, E. P.: The Treatment of Diabetes Mellitus, ed. 6, Philadelphia, 1937, Lea & Febiger.
25. Kirsner, J. B.: Parathyroids and Peptic Ulcer, Gastroenterology **34**: 145, 1954.
26. Lloyd, P.: A Case of Hypophyseal Tumor With Associated Tumor-like Enlargement of the Parathyroids and the Islets of Langerhans, Bull. Johns Hopkins Hosp. **45**: 1, 1929.
27. Louis, F., and Wills, L.: Studies in Graves Disease. Alteration in Gastric Secretion and Correlated Blood Changes, Quart. J. Med. **6**: 353, 1937.
28. McKittrick, L. S., Moore, F. D., and Warren, R.: Complications and Mortality in Subtotal Gastrectomy for Duodenal Ulcer: Report on 2-stage Procedure, Ann. Surg. **120**: 531, 1944.

29. McManus, R., and Sommers, S.: Breast Cancer Prognosis and Ovarian Cortical Stromal Hyperplasia, New England J. Med. 246: 890, 1952.
30. Moldawer, M. P., Nardi, G. L., and Raker, J. W.: Concomitance of Multiple Adenomas of Parathyroids and Pancreatic Islets With Tumor of Pituitary: Syndrome With Familial Incidence, Am. J. M. Sc. 228: 190, 1954.
31. Moore, F. D.: Endocrine Changes After Anesthesia, Surgery, and Unanesthetized Trauma in Man, Recent Progr. Hormone Res. 13: 511, 1957.
32. Moore, F. D.: Metabolic Care of the Surgical Patient, Philadelphia, 1959, W. B. Saunders Company.
33. Morse, W., Criscitiello, M., Amador, E., Renold, A., Harrison, H., Dammin, G., and Thorn, G.: Bilateral Adrenalectomy for Hypertensive Vascular Disease, Am. J. Med. 26: 315, 1959.
34. Moses, A. M.: Gastrointestinal Manifestations of Primary Hyperparathyroidism. Experience at the Mt. Sinai Hospital, N. Y., J. Mt. Sinai Hosp. 25: 339, 1958.
35. Nelson, D., Meakin, J. W., Dealy, J. B., Matson, D., Emerson, K., and Thorn, G.: A.C.T.H. Producing Tumor of the Pituitary Gland, New England J. Med. 259: 161, 1958.
36. Ostrow, D., Blanshard, D., and Gray, S. J.: Peptic Ulcer in Primary Hyperparathyroidism, Am. J. Med. In press.
37. Putnam, T. J., and Davidoff, L. M.: Acromegaly. The Pituitary Gland, Baltimore, 1938, William & Wilkins Company, chap. 42, p. 719.
38. Rothenberg, R., and Teicher, I.: Diabetes Mellitus and Peptic Ulcer, Am. J. Digest Dis. 5: 663, 1938.
39. Rowntree, L. G., and Snell, A.: Clinical Study of Addison's Disease, Philadelphia, 1931, W. B. Saunders Company.
40. Russfield, A., Reiner, L., and Klaus, H.: Endocrine Significance of Hypophyseal Tumors in Man, Am. J. Path. 32: 1055, 1956.
41. Russfield, A. B.: The Adenohypophysis, in R. C. Mellora: Analytical Pathology, New York, 1957, McGraw-Hill Book Company, Inc.
42. Sauler, H., and Zinninger, S.: Massive Islet Cell Tumor Without Hypoglycemia, Surg. Gynec. & Obst. 82: 301, 1946.
43. St. Goar, W. T.: Gastrointestinal Symptoms as a Clue to the Diagnosis of Primary Hyperparathyroidism. A Review of 45 Cases, Ann. Int. Med. 46: 102, 1957.
44. Ström, R.: A Case of Peptic Ulcer and Insulinoma, Acta chir. scandinav. 104: 252, 1952.
45. Underdahl, L., Woolner, L., and Black, B.: Multiple Endocrine Adenomata, J. Clin. Endocrinol. 13: 20, 1953.
46. Villarreal, R., Ganong, W., and Gray, S. J.: Effect of Adrenocorticotrophic Hormone Upon the Gastric Secretion of Hydrochloric Acid, Pepsin, and Electrolytes in the Dog, Am. J. Physiol. 183: 485, 1955.
47. Wilder, R. M.: Clinical Diabetes and Hyperinsulinism, Philadelphia, 1940, W. B. Saunders Company.
48. Wilson, R., Jessiman, A., and Moore, F. D.: Severe Exacerbation of Cancer of the Breast After Oophorectomy and Adrenalectomy, New England J. Med. 258: 312, 1958.
49. Wood, M.: Chronic Peptic Ulcer in 94 Diabetics, Am. J. Digest. Dis. 14: 1, 1947.
50. Zollinger, R., and Ellison, E.: Primary Peptic Ulcerations of the Jejunum Associated With Islet Cell Tumors of the Pancreas, Ann. Surg. 142: 709, 1955.

Discussion

DR. HAROLD A. ZINTEL (New York, N. Y.).—I would like to compliment Dr. Rudolf on the excellent material and his excellent presentation. This is certainly a most fascinating subject. I wanted to point out that Dr. Wermer from the College of Physicians and Surgeons has contributed a great deal to this subject. He has found that many families have multiple cases of disease in the individuals and he has suggested another approach. In other words, he suggests that there is an autosomal dominant gene, with a high degree of penetrance and variable expressivity, that produces this disease. Because of its multiple effects it is called a pleomorphic gene. I think it is most fascinating that there are so many variations or expressions presumably of this one disease. It includes other elements that Dr. Rudolf has not included—lipomas of the trachea and the bronchi, and giant gastric rugae and polypoid lesions of the gastrointestinal tract. There is one other thing I would like to mention and that is that Virginia Frantz of College of Physicians and Surgeons has suggested that histologically these tumors can be differentiated from the ordinary islet cell tumor by the fact that they have a ribbon pattern rather than one suggestive of rosettes.

DR. BERNARD FISHER (Pittsburgh, Penn.).—I, too, would like to congratulate the speaker on this excellent paper. A year ago, my brother (Edwin R. Fisher) reported a case identical with that reported today. Since that time, a brother of the reported patient died and came to autopsy, and a sister had a parathyroid adenoma removed. Both of the brothers had atrophy of the testes and we believe that it is possible that this was the trigger which set off the chain of events as described by the speaker. Examination of the pituitary showed an increased number of amphophils and basophils. An adenomatous renal hyperplasia was present in both cases. Islet cell adenoma was present. It was postulated in these cases that atrophy of the testes led to a hypertrophy of the pituitary resulting in an end-organ hyperplasia. I found this to be a most interesting paper.

DR. HENRY A. OBERHELMAN (Chicago, Ill.).—I have enjoyed Dr. Rudolf's report and would like to say that we have had 8 cases of ulcerogenic tumors in the past 2 years, 2 of which involved multiple endocrine glands. In both cases there was atrophy of the gonads. In the remaining 6 cases, the nonfunctioning islet cell tumors were located in the wall of the duodenum without pancreatic involvement, except for islet cell hyperplasia. Upon removal of the tumor from the wall of the duodenum, either by local excision or by resection, if malignant, the hypersecretion of gastric juice was abolished and peptic ulceration has not recurred. The first patient has survived 2½ years and remains asymptomatic.

DR. COLIN G. THOMAS, JR. (Chapel Hill, N. C.).—I should like to endorse the point of view and concepts submitted by the essayist and emphasize that, although the pituitary may respond to a hormone deficiency by hyperplasia, we fail to consider that the pituitary response may involve many trophic hormones rather than the one in which there is a deficiency. Although one is always hesitant to cite 1 case, it is also poor taste to present more patients than the essayist and I feel justified in giving our observations. This patient was studied primarily by Dr. Van Wyk of the Department of Pediatrics. A small goiter was noted at the age of 4 and evidence of hypothyroidism at the age of 8 as manifest by growth failure. At the age of 12 there was additional evidence of mammotropic activity, secretion from both breasts, increase in pigmentation, and profuse vaginal bleeding. A biopsy of the thyroid indicated the end-organ failure to be on the basis of chronic thyroiditis. Following the administration of triiodothyronine and the establishment of a euthyroid state, we saw remarkable recovery. The changes in the sella turcica within the course of 2 years was characterized by one initially of normal size, then increasing enlargement, and finally regression to normal. This patient then had, in response to a thyroid hormone deficiency, increased activity of mammotropic, melanotrophic, and gonadotrophic hormones accompanying enlargement of the pituitary, all of which apparently regressed following correction of the end-organ failure.

DR. ELLISON (closing).—The hypothesis presented by the authors is not only very interesting but very stimulating as well and I would like to congratulate Drs. Rudolf, Dammin, and Moore for reviving the practice of postulating a new idea or approach to a problem based on a thorough acquaintance of accumulated knowledge plus experience as clinical surgeons.

The relationship of endocrine abnormalities to acid peptic disease has been of interest to me for a long time and resulted in a review of some 20,000 consecutive autopsies recorded at the University Hospital in Columbus, Ohio, by Dr. Jerry Abrams and me. The increased incidence and severity of duodenal ulcer in patients with diabetes mellitus and the finding of adrenocortical hyperplasia and/or adenomata in over 13 per cent of the 800 and some patients with active ulcer disease only seemed to point out how little we know concerning etiology and pathogenesis of this important ailment of mankind. Failure to find evidence of an increased incidence of recognizable pituitary disease in these same ulcer patients would detract from Dr. Rudolf's postulate, however, admittedly the pathologic examination of autopsy material has at times perhaps been superficial and in turn

nonproductive of new knowledge. In addition, there is an increasing amount of experimental evidence relating at least of some control of acid secretion to the pancreas and, as pointed out by the authors, we must await identification of an initiating end-organ failure in those patients with hypersecretion, hyperacidity, and intractable ulceration associated with endocrine tumors and with tumors of the pancreatic islets in particular before adopting this idea. Incidentally, Dr. Oberhelman's experience with his 8 cases gives added weight to the postulate that the ulcerogenic factor is arising from pancreatic tumors.

A former female patient of mine, aged 30, found to have a large tumor of the right adrenal cortex but known to have been suffering from congenital virilism, presumably due to an enzyme block within the adrenal and limiting the output of cortisol (i.e., end-organ failure) and resulting in excessive production of ACTH in an attempt to stimulate further production of cortisol, might be a better example of end-organ failure resulting in pituitary overactivity and in turn tumor stimulation of the adrenal cortex.

Finally, further study is yet required to determine the predominant islet cell tumor in the associated pancreatic tumors. I believe one can safely say that they are not beta cells and appear most likely to be the delta cell first described by Bloom in 1931. With the Gomori trichrome technique, the cytoplasm of the typically cuboidal cell appears lightly basophilic, contains no granules, and is either clear or manifests a fine eosinophilic stippling. The majority of tumors studied contained a few alpha cells with the typical red granules. Our experiences with a specific delta stain, the Mallory-Heidenhain azan dye, is somewhat limited since fresh unfixed tissue is required and I would urge each of you to acquaint your pathologist with this method prior to operating on a patient suspected of harboring an islet cell tumor associated with intractable peptic ulceration. Using this method, the delta cell is found to contain dark blue granules and should be easily indentifiable.

CHANGE IN EFFECTIVE CIRCULATING VOLUME DURING EXPERIMENTAL DUMPING SYNDROME

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POSTCIBAL symptoms occurring in some patients after gastrectomy have received so much attention that the term "dumping syndrome" is a familiar one to physicians and patients alike. The etiology of this varied chain of symptoms occurring after food intake in these patients has been discussed at length and the physiologic observations that have been reported produce some confusion regarding the relative role of blood volume changes in the etiology. It is the purpose of these studies to clarify the cardiovascular response associated with the dumping syndrome. A new method for estimating changes in "effective circulating volume" has been utilized and the results obtained compared with standard methods of measurement.

Machella[4] established the fact that a shift of fluid into the bowel occurs after intralumenal introduction of hyperosmolar solution and he attributed most of the symptoms in the dumping syndrome to the local effect of this fluid shift into the bowel. Roberts and co-workers[6] then reported the observation that water shifts from the plasma occur 15 to 30 minutes after the ingestion of hyperosmolar solution by gastrectomized patients. Initially, it appeared as if most, if not all, of the dumping symptoms were directly related to the measurable change in blood volume that did occur when the small intestine was exposed to hypertonic test meals of 50 per cent glucose. A disturbing feature of this explanation as the major mechanism for the dumping syndrome in gastrectomized patients has been evidence of exaggerated autonomic response, such as the increase in digital blood flow demonstrated during the dumping episode,[1] and the increase in renal blood flow[5] noted during the symptomatic period. In addition to these confusing responses, the changes in blood volume as measured by serial determination of Evans blue (T-1824) or I^{131}-tagged albumen (RISA) have not been very well correlated with the presence or absence of symptoms. The changes have often been too small to be implicated as the only etiological agents for production of the symptom complex.

Although the cardiac output in dogs has been reported to show a marked decrease after they were fed hypertonic glucose or saline by jejunostomy or after gastrectomy,[2] our studies of plasma volume in dogs after similar jejunal

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feedings showed relatively small changes.[3] This led us to analyze our methods for estimation of plasma volume change in such experiments. These measurements have always been made by serial determination of the concentration of a previously administered indicator in the plasma, such as T-1824 or RISA. Although the deflections in the curve of this indicator usually have been interpreted as changes in plasma volume, these deflections in the late disappearance curve actually represent only changes in relative concentration of protein and water in the plasma during the period in question. The total loss of water from the plasma during the dumping episode can be estimated from these figures when they are combined with plasma volume determined initially by dilution calculations. However, if the circulating blood volume is altered by any other means than relative plasma water loss during the test, this method of measurement would not reveal this change. To detect alterations in circulating blood volume produced by actual blood or plasma loss, vasomotor changes, or vascular pooling, repeat injections of the indicator would be required to establish the dilution of the indicator in the "new" pool.

To circumvent some of the disadvantages and accumulated errors from many isolated, repeated injections of the indicator substance, we have utilized a method employing a constant infusion of Cr^{51}-tagged red blood cells associated with constant monitoring of the blood radioactivity. The rate of increase in blood radioactivity, as estimated from the "slope" of the graphic representation of this activity, can be related to the administration rate to give a value for the circulating blood volume. Changes in this effective circulating pool, whether produced by extravascular loss, temporary nonfunction by means of pooling, or contraction by pronounced vasoconstriction, would all be indicated in such an experiment by a change in slope of the graphic representation of blood radioactivity. Although not measuring a "static blood volume," a slope obtained by this constant injection method would more clearly establish the effective circulating volume during all periods of the experiment. These experiments utilized the concept described to outline the changes in effective blood volume after a test meal of hypertonic glucose solution. Prior to initiation of such experiments, the validity of the method was tested by using it to estimate the amount of blood withdrawn in simple bleeding experiments.

METHODS

For these studies, mongrel dogs, varying in weight from 10 to 20 kilograms, were used and all had been previously subjected to splenectomy. Those animals utilized in the dumping experiments were also subjected to a Roux-Y jejunal fistula just below the duodenal-jejunal flexure. The nonfunctional limb of this jejunostomy was approximately 15 inches in length to prevent loss of intestinal contents from the external opening, and the jejunostomy was utilized for intrajejunal administration of hypertonic glucose solution during the experiments to be described.

The radioactive indicator was administered by a motor-driven syringe (0.21 c.c. per minute), and through a catheter placed surgically in a peripheral vein. The indicator utilized in our initial experiments was I^{131}-tagged albumen (RISA) but red blood cells tagged with Cr^{51} were found to be much more satisfactory for these studies, and were utilized in all but the initial attempts. To maintain mixing of the red cells in the syringe, an agitator that utilized a windshield-wiper mechanism was employed (Fig. 1).

The radioactivity of the circulating blood was monitored continuously by an arterially fed plastic coil placed in a well-type scintillation counter. This coil was placed in the femoral artery so that the inflow to the coil was inserted proximally and the outflow was drained into the distal limb of the incised artery. The counts recorded by the analytic count rate meter were fed into a direct-writing graphic recorder. The graphic representation of the radioactivity in the coil was calibrated by withdrawing several samples of arterial blood during the experiment and determining the activity of a known volume of blood with a scintillation detector-scaler circuit. Heparin administered during the entire course of the experiment was used for anticoagulation. Anesthesia with Nembutal was utilized in many experiments testing the methods described but general anesthesia was not utilized in the experiments studying the dumping syndrome. All vascular polyethylene catheters were inserted under local infiltration anesthesia.

Blood volume by standard dilution technique was first determined by means of 3 control samples at 10-minute intervals after initial injection of a known amount of the isotope. The graphic recording was calibrated during this period. The general plan of the experiments was to establish a slope of blood radioactivity in the dog during a constant injection control period of 30 minutes. The animal was then subjected to blood withdrawal or intrajejunal administration of hypertonic glucose, while the constant infusion and recording of blood radioactivity continued. Hematocrit determinations were made at intervals during the course of the experiment.

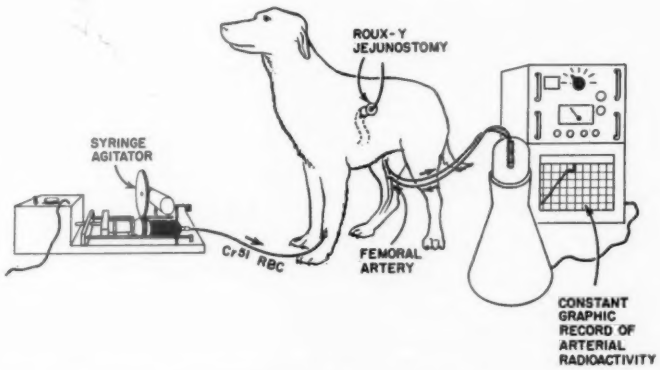

Fig. 1.—Diagram of experimental preparation for determining alterations in effective circulating volume.

Calculation of total blood volume by the dilution method was made by the standard formula: concentration A × volume A = concentration B × volume B. Total blood volume measured by the constant infusion technique was calculated for any given period with the following formula: total blood volume = total counts infused per minute divided by change in arterial counts per minute per cubic centimeter. Because of minor variations in the slope over short periods, all calculations using this latter method were limited to periods of 15 minutes or longer. Change in blood volume could be expressed as a percentile change by comparing only the change in counts per minute during 2 periods being compared if the infusion rate was constant throughout. There was an inverse relationship between the change in counting rate (or "slope") and the blood volume as expressed by this formula:

$$\frac{\text{Blood volume B}}{\text{Blood volume A}} = \frac{\Delta \text{ c.p.m. A}}{\Delta \text{ c.p.m. B}}$$

RESULTS

1. With the use of either RISA or Cr^{51}-tagged red blood cells, the constant infusion of isotope produced a steady increase in blood radioactivity when measured in the arterial coil. The slope of the graphic recording of arterial

TABLE I. CALCULATION OF TOTAL BLOOD VOLUME BY CONSTANT INFUSION OF Cr^{51} COMPARED WITH SINGLE INJECTION METHOD

DOG. NO.	TOTAL BLOOD VOLUME (Cr^{51})		
	SIMPLE DILUTION METHOD (C.C.)	CONSTANT INFUSION METHOD (C.C.)	PER CENT DIFFERENCE
C736	1,634	1,699	+ 3.8
C903	1,042	1,229	+17.9
C807	853	1,150	+34.8
C986	1,184	1,090	− 7.9
C776	657	709	+ 7.9
C999	1,063	1,190	+11.9
C905	786	891	+13.4
C391	2,002	2,119	+ 5.8
C820	946	1,193	+26.2
C745	686	957	+39.5
C7	1,285	1,258	− 2.0
C947	1,026	1,166	+14.2
D2	1,108	876	−30.0
D15	1,493	1,693	+13.4
C534	1,109	1,182	+ 6.6
C817	1,194	1,330	+11.4
D18	1,601	1,861	+16.2
D77	1,459	1,558	+ 6.8
D108	1,777	1,855	+ 4.4
D64	1,380	1,575	+14.2
C995	1,296	1,599	+23.4
C991	1,085	1,224	+12.8
Mean			+11.1%

TABLE II. EVALUATION OF THE ACCURACY OF THE CONSTANT INFUSION METHOD BY COMPARISON OF RESULTS WITH KNOWN CHANGES IN CIRCULATING BLOOD VOLUME PRODUCED BY MEASURED HEMORRHAGE (DOGS)

ISOTOPE	BLOOD VOLUME BEFORE HEMORRHAGE (C.C.)	MEASURED VOLUME WITHDRAWN		ESTIMATED DECREASE IN BLOOD VOLUME		DIFFERENCE	
		C.C.	PER CENT TOTAL BLOOD VOLUME	C.C.	PER CENT TOTAL BLOOD VOLUME	C.C.	PER CENT TOTAL BLOOD VOLUME
Cr^{51}	1,699	240	14.1	267	15.7	+27	1.6
Cr^{51}	1,229	190	15.5	131	10.7	−59	4.8
Cr^{51}	1,150	200	17.4	259	22.5	+59	5.1
Cr^{51}	1,090	250	22.9	219	20.1	−31	2.8
Cr^{51}	709	180	25.4	147	20.7	−33	4.7
Cr^{51}	1,190	220	19.5	289	24.3	+69	4.8
Cr^{51}	891	215	24.1	202	22.7	−13	1.4
Cr^{51}	2,119	450	21.2	548	25.9	+98	4.7
RISA	1,558	300	19.3	279	17.9	−21	1.4
RISA	1,603	275	17.2	350	21.8	+75	4.6
Mean							3.6%

TABLE III. SERIAL CHANGE IN TOTAL BLOOD VOLUME BY CONSTANT INFUSION TECHNIQUE (Cr^{51}) AFTER INTRAJEJUNAL ADMINISTRATION OF 150 C.C. 50 PER CENT DEXTROSE

DOG NO.	CONTROL TOTAL BLOOD VOLUME (C.C.)	CHANGE IN BLOOD VOLUME (AS PER CENT OF CONTROL)			
		1ST 15 MINUTES	1ST 30 MINUTES	30-60 MINUTES	60-90 MINUTES
108	1,855	28.5↓	20.9↓	6.1↓	2.9↑
64	1,515	18.2↓	17.1↓	9.6↓	3.3↓
995	1,599	19.4↓	17.5↓	20.3↓	6.8↓
991	1,224	27.2↓	14.0↓	3.3↓	2.6↑

radioactivity tended to gradually decrease with time when RISA was used. Cr^{51}-tagged red blood cells were therefore used for all but the initial experiments and this change in slope was minimal.

2. The total blood volume as measured by the standard dilution method was compared with values obtained immediately thereafter by the constant infusion method. The data obtained using Cr^{51}-tagged red blood cells are shown in Table I and each calculation is based on time periods of approximately 30 minutes. In 19 of these 22 experiments, the volume estimated from the constant infusion method was larger than that measured by the single injection technique. This increase over the values obtained by the standard technique ranged from 3.8 to 39.5 per cent but the mean difference was 11.1 per cent.

3. Sudden withdrawal of approximately 20 per cent of the total blood volume (as measured by constant infusion technique) was carried out in 10 experiments. The measured volume removed was compared with the defect in blood volume detected by the change in the graphic recording. It can be seen in Table II that approximation of blood loss by this infusion technique was in relatively good agreement with the known volume withdrawn. The discrepancy noted between the known deficit and the calculated deficit was 13 to 98 c.c. and this difference represented 1.4 to 5.1 per cent of the total blood volumes of these dogs. The mean difference between the final blood volume estimated with this technique and the original blood volume minus the measured hemorrhage was 3.6 per cent.

4. After these initial experiments to evaluate the method, 14 dumping experiments were carried out with the constant infusion method in dogs that had previously been subjected to splenectomy and Roux-Y jejunal fistula. The serial change in total blood volume was estimated from the graph of arterial radioactivity before and after intrajejunal administration of 150 to 200 c.c. of 50 per cent glucose solution. For our calculations we measured the slope of arterial radioactivity for the following time periods: (1) 15- to 30-minute control period, (2) the initial 15 minutes after glucose administration, (3) the initial 30 minutes after glucose, (4) 30 to 60 minutes after glucose, and (5) 60 to 90 minutes after introduction of the glucose solution. A schematic diagram of a typical experiment is shown in Fig. 2. The serial changes in blood volume as measured by this approach in 4 of the dogs are shown in Table III. The maximum decrease in blood volume in these experiments occurred in the first 15-minute period, was usually returning toward normal in the second 30-minute period, and reached the control values for blood volume after 1 hour. The decrease in effective blood volume measured in the first 15 minutes after intrajejunal administration of glucose solution in the entire group of dogs studied is shown in Table IV. The range of decrease in effective blood volume was 11.2 to 38.0 per cent and for these 14 experiments the mean decrease was 23.1 per cent. If the 3 experiments in which the dogs received 200 c.c. of hypertonic glucose are eliminated, the mean decrease in blood volume in the first 15 minutes was 20.1 per cent.*

*There was constant measurement of arterial pressure in 4 experiments. Small decrease in blood pressure occurred 15 to 30 minutes after administration of intrajejunal glucose in 3 of these experiments. The greatest change observed was a decrease from 170 to 140 mm. Hg.

The change in red cell mass and plasma volume were calculated from the changes in total blood volume by means of serial hematocrit determinations. The mean decrease in red cell mass in the first 15 minutes after 150 c.c. intrajejunal glucose was 15.7 per cent (10 experiments). The mean decrease in plasma volume in these same experiments was 24.4 per cent.

TABLE IV. DECREASE IN EFFECTIVE CIRCULATING BLOOD VOLUME IN THE FIRST 15 MINUTES AFTER INTRAJEJUNAL ADMINISTRATION OF 50 PER CENT GLUCOSE SOLUTION (USING CONSTANT INFUSION TECHNIQUE FOR BLOOD VOLUME)

DOG NO.	ISOTOPE	VOLUME GLUCOSE INTRODUCED INTO JEJUNUM (C.C.)	CONTROL TOTAL BLOOD VOLUME (C.C.)	DECREASE IN TOTAL BLOOD VOLUME IN 1ST 15 MINUTES	
				C.C.	PER CENT
918	RISA	200	2,751	810	29.4
947	Cr^{51}	200	1,166	443	38.0
534	Cr^{51}	200	1,182	418	35.4
9	RISA	150	1,406	315	22.4
845	Cr^{51}	150	1,227	125	12.4
2	Cr^{51}	150	876	124	14.2
15	Cr^{51}	150	1,693	325	19.2
817	Cr^{51}	150	1,330	249	11.2
18	Cr^{51}	150	1,861	372	20.0
77	Cr^{51}	150	1,558	440	28.3
108	Cr^{51}	150	1,855	528	28.5
64	Cr^{51}	150	1,515	279	18.2
995	Cr^{51}	150	1,599	309	19.4
991	Cr^{51}	150	1,224	333	27.2
Mean					23.1%

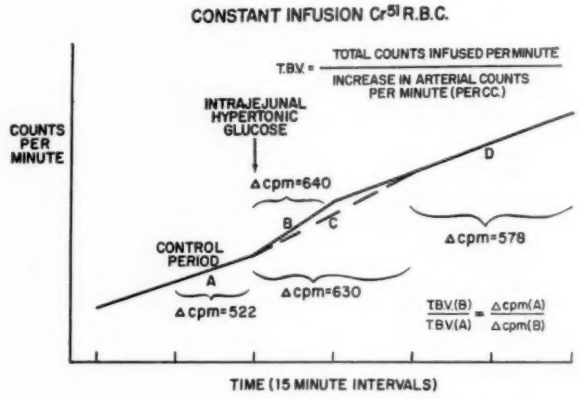

Fig. 2.—Schematic representation of data obtained with the constant Cr^{51} infusion method (dog subjected to intrajejunal 50 per cent glucose solution).

DISCUSSION

The method utilized for measuring change in effective blood volume in these experiments represents a modification of the standard dilution method. The product of the increment in blood radioactivity and the volume of distribution of the active circulating pool during a specific time period can be equated to the known activity of the indicator administered during this interval. Thus the calculations are similar to those used with the single injection technique (concentration A × volume A = concentration B × volume B). The failure of a small portion of the administered isotope to attain complete "mixing" is a small but definite error inherent in this method. However, this is

minimized if the time periods employed for the calculations are of sufficient length. It should be also noted that the time necessary for the rate meter to equilibrate caused a lag behind the true arterial counting rate at any given moment. With the 15- to 30-minute time intervals employed this small delay introduces an insignificant error into the measurement of blood volume. The recorded curve is parallel to the theoretical curve and, hence, has an identical slope.

The recorded counting rate during these experiments varied from 10^4 c.p.m. following the initial single injection to 10^5 c.p.m. at the end of the experiment. With the rate meter time constants used in these experiments, the probable error of measurement of the counting rate varied from 1.15 to 0.16 per cent.

When the blood volume obtained by the constant infusion method was compared with that from a single injection of the Cr^{51}-tagged red cells there was marked variability. Although the values obtained using this new method were sometimes significantly lower than those obtained with the standard dilution method, the mean value for all determinations with the constant infusion method was slightly higher than the mean value obtained by standard dilution techniques (11.1 per cent greater). When the determination of blood volume by this technique was compared with the measured amount of blood withdrawn in simple bleeding experiments the agreement was surprisingly good. From these initial studies we felt that this was a valuable technique for estimating serial changes in effective blood volume during acute circulatory readjustment situations. The often confusing circulatory dynamics of experimental dumping syndrome appeared worthy of study by this new approach.

After intrajejunal administration of hypertonic material there was a marked alteration in the graph of arterial radioactivity. The increase in slope of Cr^{51} activity during the first 15 minutes after the glucose meal was given represented a 11.2 to 38 per cent decrease in effective circulating volume. If the net change in volume was calculated from the data obtained over the first 30 minutes after the test meal, the change in effective circulating volume was less marked. Further return toward the control slope then occurred in subsequent time periods. These circulatory changes observed in the experimental animal do coincide chronologically with the period of symptoms in patients exhibiting the dumping syndrome.

We have also measured the deflection in the late "fall-off" curve of T-1824 or RISA after introduodenal or intrajejunal administration of hypertonic glucose solution in dogs.[3] The losses recorded with this indirect method were actually "relative water losses" and not true measurements of plasma volume. Using the late disappearance curve of Cr^{51}-tagged red cells and simultaneous hematocrits, we also detected an acute shift of water from red cells. The water loss from both plasma and red cells in experiments, where both measurements were carried out, was of much smaller magnitude than the circulatory alterations described here. The mean value for maximum water loss expressed as a proportion of the blood volume in these previously reported experiments was only 9.3 per cent. These data are compared with data from experiments using the constant infusion method in Fig. 3. It would appear that a shift of water

from the blood into the bowel must occur after administration of a hypertonic test meal. This loss, however, is only partially responsible for the acute decrease in effective circulating volume and other factors must also play an important role in this cardiovascular response.

A consideration of factors responsible for such an acute and transient decrease in effective circulating volume must stress redistribution of the blood volume or "pooling." A relatively stagnant area of flow or a predominance of flow within a less active circuit could effect such a transient decrease in effective circulating volume. Confusing alterations in renal blood flow and digital blood flow that have been reported with dumping may also be a manifestation of this acute redistribution of flow. Another factor that may play a role in our acute changes in effective circulating volume is an exaggerated vasomotor response. Administration of vasopressor agents has been shown to decrease blood volume.[7] Using the constant infusion method, we have also produced a pronounced increase in slope (or decrease in effective circulating volume) when a vasopressor agent was infused. It is also apparent from clinical studies of the dumping syndrome that this vasomotor factor may play a large role in the obvious circulatory alteration.

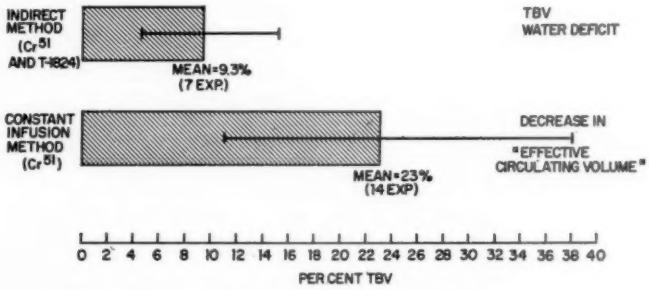
INDIRECT METHOD (Cr51 AND T-1824)
MEAN=9.3% (7 EXP.)
TBV WATER DEFICIT
CONSTANT INFUSION METHOD (Cr51)
MEAN=23% (14 EXP)
DECREASE IN "EFFECTIVE CIRCULATING VOLUME"
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
PER CENT TBV

Fig. 3.—Blood volume change after intrajejunal administration of 50 per cent glucose solution. The indirect method, which measures only relative water loss, is compared with these data obtained with the constant infusion method (Cr^{51}).

From these studies we believe that acute alterations in the available blood volume do occur soon after intrajejunal administration of a hypertonic test meal. The usual methods for determining plasma or blood water loss with the dumping syndrome reveal only a fraction of the loss from the functional blood volume, however. Confirmation of these studies in patients must await refinement of the method described since the level of radioactivity required in these experiments was unduly high for routine clinical use.

SUMMARY

Confusion regarding the significance of blood volume alterations in postgastrectomy dumping syndrome prompted studies with a heretofore undescribed method for measuring acute changes in effective circulating volume. In these studies on dogs, constant graphic recording of arterial radioactivity accompanied a carefully controlled constant infusion of Cr^{51}-tagged red blood cells. Acute changes in effective circulating volume were detected and measured by the changes in slope of the arterial radioactivity. After evaluation of the method by experiments with measured hemorrhage, this technique was applied

to dogs before and after intrajejunal administration of hypertonic glucose solution. Acute alterations in effective circulating volume were significantly greater than the changes previously noted in similar experiments in which the classic "indirect" method was employed.

The circulatory alteration with this experimental situation was therefore greater than could be accounted for by intralumenal water loss alone, and a redistribution of blood flow is considered the most likely explanation. The method utilized in these experiments deserves further study for application to other problems, and possible clinical use.

We are indebted to R. Barters, S. Denacks, M. Hood, and F. Washington for invaluable technical assistance.

REFERENCES

1. Hinshaw, D. B., Joergenson, E. J., Davis, H. A., and Stafford, C. E.: Peripheral Blood Flow and Blood Volume Studies in the Dumping Syndrome, A. M. A. Arch. Surg. 74: 686-693, 1957.
2. Klauber, L. D., Poppell, J. W., Kahn, R. E., Randall, H. T., and Roberts, K. E.: Alterations in Cardiac Output Following the Intrajejunal Administration of Hypertonic Solutions, A. M. A. Arch. Int. Med. 100: 225, 1957.
3. Lawrence, W., Jr., and Mathews, D. H.: A Study of Water "Shifts" in Experimental Dumping Syndrome, S. Forum 10: 180, 1959.
4. Machella, T. E.: The Mechanism of the Post-gastrectomy Dumping Syndrome, Ann. Surg. 130: 145, 1949.
5. Morris, G. C., Jr., Greenfield, L. J., and Jordan, G. L., Jr.: Alterations in Renal Hemodynamics in Patients With the "Dumping Syndrome," S. Forum 8: 202, 1957.
6. Roberts, K. E., Randall, H. T., Farr, H. W., Kidwell, A. P., McNeer, G. P., and Pack, G. T.: Cardiovascular and Blood Volume Alterations Resulting From Intrajejunal Administration of Hypertonic Solutions to Gastrectomized Patients; the Relationship of These Changes to the Dumping Syndrome, Ann. Surg. 140: 631-640, 1954.
7. Thistlethwaite, J. R., Spencer, W. A., and Albert, S. N.: Blood Volume Fluctuations Determined by Radioisotopes of Chromium and Radioactive Iodinated Serum Albumin, Surg. Gynec. & Obst. 105: 34, 1957.

Discussion

DR. RAYMOND C. READ (Minneapolis, Minn.).—I would like to thank Dr. Hay and Dr. Randall for the privilege of discussing this paper. At the Minneapolis Veterans Hospital, we have found that there is a consistent increase in pulse pressure in the dumping syndrome. This finding, coupled with the other evidence presented today, is in agreement with the idea that the essential vasomotor state in the dumping syndrome is one of vasodilatation. We were intrigued as to the mechanism of this response. The blood pressure findings were reminiscent of the classic work of Soma Weiss on the effects of acetylcholine in man. It was of interest therefore to find that the infusion of 1 ml. per kilogram of body weight of 50 per cent glucose solution into the dog produced typically a vasomotor reaction which was practically identical to that obtained with 5 mcg. of acetylcholine per kilogram body weight above and below 6 mcg. per kilogram. This slide is taken from observations made in a vagotomized animal. I want you to notice that there is bradycardia produced by glucose after vagotomy and that this slowing is similar to what happens with acetylcholine. The response is fairly rapid as one can see from the time scale and in all instances there is evidence for vasodilatation as witnessed by the increased pulse pressure and the loss of the dicrotic notch in the pressure pulse. The threshold for this phenomenon with hypertonic solutions is a change in the osmolarity of the plasma. These data were obtained in the totally perfused dog at constant flow using various hypertonic media including 50 per cent glucose and various salt solutions. The threshold is an increase in plasma osmolarity of about 10 per cent which is within the range of change seen in the dumping syndrome.

We therefore believe that the dumping syndrome represents the effect of changes in hypertonicity on the bowel and other parts of the body. This action is mediated in a manner which resembles the local release of acetylcholine.

DR. THOMAS T. WHITE (Seattle, Wash.).—I have been much interested in this work. One of the things which interested me was the work done in Japan by Terada, which basically shows that there is a great increase in histamine production from the small intestine with hypermotility and, apparently, some of the Japanese investigators feel that the vasodilatations described in the fingers and the kidneys might be on this basis—on the basis of the release of histamine—rather than some other phenomenon. It was reported earlier this year by O'Hara that serotonin was released in large amounts into the small intestine when hypertonic solutions were placed in the small intestine. They apparently believe that histamine is also released in large amounts if hypertonic solutions are introduced into the small intestine.

DR. LAWRENCE (closing).—I would like to thank the discussors for their comments. In regard to the vasomotor response phenomenon, I am sure that it is a complex series of events that must occur with the dumping syndrome—much more complex than we initially realized. This is further shown by the fact that when we put hypertonic solution in the bowel, we get a profound decrease in pancreatic secretion which is in the range of the decrease one gets with Adrenalin, despite an insignificant change in blood pressure. We actually tried to obliterate this by adrenalectomy but couldn't. I want to express the wish that others attempt this general approach to evaluate changes in effective volume by a constant infusion technique for study of some of the other acute surgical problems to which it might be applied. At present we have developed it only to a crude state. I hope others try this method as we have been rather intrigued by its possibilities.

STUDIES ON THE ROLE OF PANCREATIC AND BILIARY SECRETIONS IN THE MECHANISM OF GASTRIC INHIBITION BY FAT

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EWALD and Boas[1] first described the inhibition of gastric motor activity which occurs when fat is present in the upper small intestine. A similar inhibitory effect of fat on gastric secretion was observed in Pavlov's laboratory.[8] In 1906 Lonnquist[12] showed that gastric activity was inhibited only when fat was in the upper small intestine. Fat in the stomach did not alter gastric secretion or motility. The main inhibitory effect of fat on gastric secretion and motility is generally thought to originate in the duodenum.[18] Pavlov and his coworkers[20] attributed this inhibition to a nervous reflex between the duodenum and the stomach. However, the classic experiments of Farrell and Ivy,[2] demonstrating secretory and motor inhibition by fat of a totally denervated autotransplanted pouch, suggested the participation of a humoral mechanism. The recognition of a humoral mechanism mediating gastric inhibition by fat led to testing of bowel extracts. Such extracts prepared from canine small bowel mucosa were found to inhibit Heidenhain pouch secretion. It was postulated that these extracts contained a hormonal substance which was called "enterogastrone."[11] According to the now classic conception of gastric inhibition by fat, the mucosa of the proximal small bowel, when in contact with a sufficient concentration of fat, presumably releases this inhibitory substance.[6] Intravenous injections of purified enterogastrone preparations to dogs have been shown to inhibit gastric secretion stimulated by feeding, insulin, histamine, or pilocarpine.[4, 7] However, exogenous enterogastrone does not inhibit the motility of a denervated gastric pouch,[10] whereas fat in the proximal small intestine will have an inhibitory effect under these conditions. Enterogastrone has little, if any, effect on gastric secretion of human beings.[9] Enterogastrone has no effect on gastric secretion in the rat,[17] although fat is a very effective inhibitor of gastric secretion in this animal.

Although there is little doubt that extracts of bowel mucosa inhibit gastric secretion in dogs, the assumption that such extracts contain the specific principle mediating gastric inhibition by fat appears unwarranted. Our interest in this problem was stimulated by the finding that parenterally administered bile salts inhibited gastric secretion in rats.[15] This suggested the possibility that bile salts could be the agent mediating gastric inhibition by fat, since bile

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*John and Mary Markle Scholar in Medical Science.

salts are reabsorbed during fat absorption. Therefore, the purpose of this study was to determine the relationship of the presence or absence of bile salts in the intestine to gastric inhibition by fat. Pancreatic lipase was similarly studied because of its important role in fat digestion.

MATERIALS AND METHODS

Data are presented on experiments carried out on 206 male Holtzman rats weighing from 160 to 220 grams. All of the animals originated from the same colony.*

Role of Bile Salts in Gastric Inhibition by Fat.—Gastric secretion was measured by the pylorus ligation method[19] in 130 rats. Prior to pyloric ligation, the animals were fasted for 48 hours in individual cages with wide-mesh wire bottoms. Water was given ad libitum during the fasting period. The rats were divided into 5 groups of paired control and treated rats. In all rats except those of Group I, the proximal bile duct was shunted to the rectum 24 hours prior to pyloric ligation by a technique described previously.[15] With this method the entire bile output is diverted from the small intestine and cecum, and pancreatic secretions which reach the duodenum via the distal portion of the bile duct are left undisturbed. It was established previously that diversion of bile from the small intestine does not per se alter gastric secretion in the rat.[15] In rats belonging to Group I, a sham operation was performed. Pyloric ligation was performed in all rats under light ether anesthesia. Simultaneously, the treated rats received the following intraduodenal injections: Groups I (15 rats) and II (19 rats); 2 ml. of olive oil each; Group III (13 rats); 2 ml. of olive oil and 400 mg. of Tween 80; Group IV (10 rats); 2 ml. of olive oil and 30 mg. of bile salts;† Group V (8 rats); 30 mg. bile salts in 2 ml. of normal saline. Doses were on basis of 200 mg. of rat weight and were administered by a single injection through a 26 gauge needle inserted into the duodenum immediately distal to the pyloric ligature. The paired random controls received the same volume of normal saline. Six hours later the rats were killed and their stomachs removed. The gastric contents were studied individually for volume and free hydrochloric acid concentration. The 6-hour output of free hydrochloric acid in milliequivalents was calculated for each animal. The mean values for each group were then compared with the mean values for the respective paired control animals.

Role of Pancreatic Lipase in Gastric Inhibition by Fat.—Originally, attempts were made to shunt both pancreatic juice and bile away from the small intestine by cannulation of the distal portion of the bile duct. However, the animals did not tolerate this combined loss. Therefore, the principle of the Thiry-Vella loop was adopted in order to obtain a segment of bowel relatively free of lipase and free of bile. Thiry-Vella loops, 5 inches in length, were constructed from the proximal small bowel in 76 rats. Pyloric ligation following a 48-hour fast was performed 2 weeks later. By random selection the rats were divided into 4 groups. In Group I (16 rats), which served as a control group, 1 ml. of saline was injected into the Thiry-Vella loop simultaneously with pyloric ligation. Both ends of the loop were then ligated to prevent egress of its contents. The other groups were treated as follows: Group II (16 rats); 1 ml. of olive oil; Group III (9 rats); 1 ml. of an artificial oil emulsion, Lipomul; Group IV (19 rats); 1 ml. of olive oil and 30 mg. of bile salts; Group V (16 rats); 1 ml. of olive oil, 30 mg. of bile salts, and 5 mg. of lipase. Doses were on the basis of 200 Gm. of rat weight and were instilled into the Thiry-Vella loop in a single injection. Six hours after pyloric ligation the rats were killed and the gastric contents studied as in the other series. The mean 6-hour output of free hydrochloric acid in milliequivalents of each test group was then compared to that of control Group I.

RESULTS

Role of Bile Salts in Gastric Inhibition by Fat.—The results are summarized in Fig. 1. In the intact rats of Group I given olive oil, there was a

*Holtzman Rat Co., Madison, Wis.
†Ox bile salts, Nutritional Biochemicals Corporation.

mean inhibition of 85 per cent in the 6-hour output of free hydrochloric acid as compared to the paired controls. The same amount of oil caused only 15 per cent inhibition in the bile-shunted rats of Group II, and in the rats of Group III which received oil and Tween 80, the mean inhibition was only 12 per cent. However, 88 per cent inhibition occurred in the bile-shunted rats of Group IV

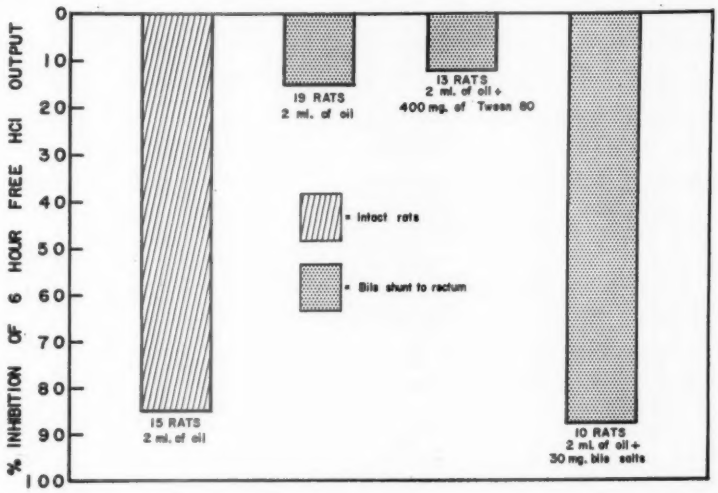

Fig. 1.—Gastric secretion in rats. Relation of presence or absence of bile salts in small intestine to inhibition of secretion by fat in the intestine. Bars represent per cent inhibition in 6-hour output of free hydrochloric acid as compared to the paired controls. From left to right, Groups, I, II, III, and IV.

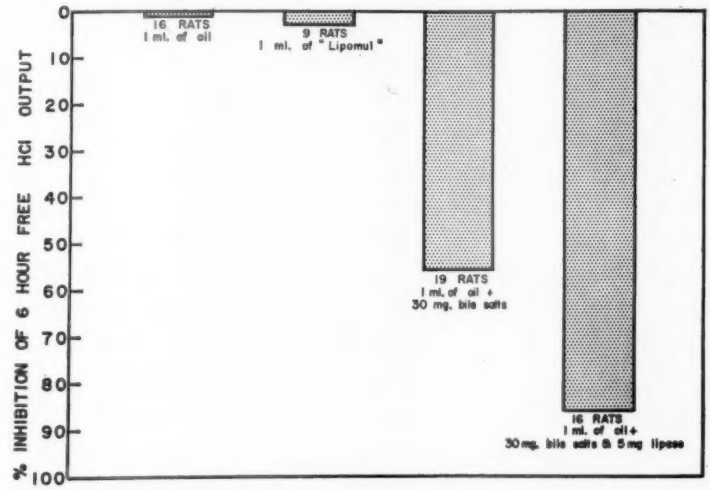

Fig. 2.—Gastric secretion in rats. Relation of bile salts and lipase to inhibition of secretion by fat in a Thiry-Vella loop. Bars represent per cent inhibition in 6-hour output of free hydrochloric acid as compared to control group. From left to right, Groups II, III, IV, and V.

which received oil and bile salts. In the rats of Group V there was essentially no change. A 9 per cent increase in gastric secretion occurred in the rats receiving bile salts alone intraduodenally. In only Groups I and IV was the mean output of hydrochloric acid significantly different from that of the paired controls.

Role of Pancreatic Lipase in Gastric Inhibition by Fat.—The results of the second series of experiments are summarized in Fig. 2. The total outputs of

free hydrochloric acid in Groups II, III, IV, and V were compared to control Group I. In rats of Group II, receiving 1 ml. of olive oil, and in rats of Group III which received 1 ml. of Lipomul, the mean deviation from the control group was –2 and –3 per cent, respectively. The rats of Group IV which received 1 ml. of oil mixed with 30 mg. of bile salts had a mean inhibition of 56 per cent. A mean inhibition of 86 per cent occurred in the rats of Group V, in which the mixture of oil, bile salts, and lipase was instilled into the loop. Only in this latter group was there visible evidence of fat absorption. In only Groups IV and V was the mean output of hydrochloric acid significantly different from that of control Group I. Also, the means of these 2 groups were significantly different ($p < 0.001$).

DISCUSSION

The main conclusion drawn from the data is that inhibition of gastric secretion by fat does not occur in the absence of adequate amounts of bile salts and lipase. The results obtained in Group IV in the second experimental series would suggest that some inhibition can occur in the absence of lipase. However, a small amount of lipase is known to be present in succus entericus, and indeed in several of the animals of Group IV the contents of the loop were found to be emulsified when the animals were sacrificed. The classic theory of gastric inhibition by fat postulates a release of enterogastrone when the intestinal mucosa is in contact with fat. Our data indicate that a stable emulsion of fat is necessary in order for gastric inhibition by fat to take place. Since only a stable emulsion of fat, that is, the emulsion occurring when adequate concentrations of bile salt and lipase are present, is absorbable, the logical inference is that gastric inhibition by fat is related to the process of fat absorption. However, the products of fat digestion, such as those contained in lymph, have been shown not to alter gastric secretion when administered intravenously.[10, 11] Similarly, we observed that intravenously injected fat emulsions had no effect on gastric secretion in the rat. On the other hand, bile salts are reabsorbed at a more rapid rate during fat absorption. Previously, we had found that bile salts administered parenterally to rats and dogs inhibited gastric secretion and also inhibited the in vitro secretory activity of the frog stomach.[15, 16] Therefore, it is tempting to speculate that gastric inhibition by fat is mediated by reabsorbed bile salts. The data reported herein neither prove nor disprove this speculation.

Another possible mechanism is the increase in osmolarity of intestinal contents resulting from fine emulsification of fat. Work under way in our laboratory has shown that under conditions of increased intestinal osmolarity, there is an increase in intestinal blood flow and a concomitant decrease in gastric blood flow.[14]

The relationship of pancreatic and biliary secretions to gastric secretory activity has been thought to be merely that of a buffer reducing acidity of duodenal contents. Apparently their role may be a more complex one. Recently, Greenlee, Nelsen, and Dragstedt[5] demonstrated an increase in gastric secretion in dogs following construction of total pancreatic fistulas. A similar

effect was reported recently after total pancreatectomy in dogs.[3] Martin and co-workers found that duodenal ulcers did not develop in dogs with internal biliary and pancreatic shunts to the ileum if the length of terminal ileum between the transplanted ducts and ileocecal valve was greater than 75 cm. Under these conditions protection from duodenal ulceration occurred even though any possibility of buffering of hydrochloric acid by bile and pancreatic juice was precluded.[13] Gastric inhibition by fat probably plays a role in the over-all regulation of gastric secretory activity. Impairment of this intestinal inhibitory phase could lead to increased gastric secretory activity. Our findings, indicating that such an impairment of gastric inhibition by fat occurs if bile or pancreatic juice are absent from the small intestine, may explain the increased gastric secretion observed after creation of pancreatic fistulas or pancreatectomy. Our findings may also explain the results of Martin and colleagues. It seems reasonable to assume that in the dogs with a longer segment of bowel containing bile and pancreatic juice, sufficient digestion of fat, and consequently gastric inhibition, occurred to curb hydrochloric acid secretion thereby obviating ulcer formation.

SUMMARY

Gastric secretion was measured in 206 rats by the pylorus ligation method. The introduction of oil into the duodenum at time of pyloric ligation caused 85 per cent inhibition of 6-hour gastric secretion as compared to paired controls. If bile had been diverted previously from the small intestine, the same volume of oil alone, or oil mixed with an artificial emulsifier produced only minor inhibition of secretion. The latter was suppressed only when bile salts were added to the administered fat. In rats prepared with a Thiry-Vella loop (devoid of pancreatic and biliary secretions) maximal inhibition of gastric secretion occurred only when a stable, that is, absorbable emulsion (oil plus bile salts plus lipase), was introduced into the loop. Oil alone was without effect, oil mixed with bile salts caused only 56 per cent inhibition, and oil mixed with bile salts and lipase produced 86 per cent inhibition. Quantitation of inhibition of gastric secretion under these various conditions by the different test solutions was accomplished by comparison with control groups in which saline alone was injected into the bowel.

In conclusion, gastric inhibition by fat is linked to the process of fat absorption and bile salts, which are reabsorbed with fat, may represent the specific agent mediating gastric inhibition by fat.

REFERENCES

1. Ewald, C. A., and Boas, J.: Beiträge zur Physiologie und Pathologie der Verdauung, Virchows Arch. path. Anat. **104**: 271-305, 1886.
2. Farrell, J. E., and Ivy, A. C.: Studies on the Motility of the Transplanted Gastric Pouch, Am. J. Physiol. **76**: 227, 1926.
3. Goelzer, R. E.: Effect of Total Pancreatectomy on Gastric Secretion, S. Forum **10**: 167, 1959.
4. Gray, J. S., Bradley, W. B., and Ivy, A. C.: On the Preparation and Biological Assay of Enterogastrone, Am. J. Physiol. **118**: 463-476, 1937.
5. Greenlee, H. B., Nelsen, T. S., and Dragstedt, L. R.: The Effect of Pancreatic Fistula on Gastric Secretion, A. M. A. Arch. Surg. **79**: 156, 1959.
6. Grossman, M. I.: Gastrointestinal Hormones, Physiol. Rev. **30**: 33-90, 1950.

7. Grossman, M. I., Greengard, H., Wooley, J. R., and Ivy, A. C.: Pepsin Secretion and Enterogastrone, Am. J. Physiol. **141**: 281, 1944.
8. Khigine, P.: Activité sécrétoire de l'estomac du chien, Arch. sc. biol. **3**: 461, 1894-1895.
9. Kirsner, J. B., Levin, E., and Palmer, W. L.: Effect of Dialyzed Enterogastrone Upon Twelve Hour Nocturnal Gastric Secretion in Man, Proc. Soc. Exper. Biol. & Med. **70**: 685, 1949.
10. Kosaka, T., and Lim, R. K.: On the Mechanism of Inhibition of Gastric Motility by Fat. An Inhibitory Agent From the Intestinal Mucosa, Chinese J. Physiol. **7**: 5, 1933.
11. Kosaka, T., Lim, R. K., Ling, S. M., and Liu, A. C.: On the Mechanism of the Inhibition of Gastric Secretion by Fat. A Gastric Inhibitory Agent Obtained From the Intestinal Mucosa, Chinese J. Physiol. **6**: 107, 1932.
12. Lonnquist, B.: Beitrage zur Kenntnis der Magensaftabsonderung, Skandinav. arch. physiol. **18**: 194, 1960.
13. Martin, K. A., Beal, J. M., and Glenn, F.: The Minor Role of Hydrochloric Acid in Experimental Production of Peptic Ulcer in Dogs, Gastroenterology **33**: 631, 1957.
14. Menguy, R.: Unpublished data.
15. Menguy, R., and Koger, E.: Mechanism of Inhibition of Gastric Secretion in the Rat Following Bile Duct Ligation, Proc. Soc. Exper. Biol. & Med. **101**: 666, 1959.
16. Menguy, R., and Peissner, L.: In Vivo and In Vitro Effects of Bile Salts on Gastric Secretion, Clin. Res. **8**: 50, 1960.
17. Morris, C. R., Grossman, M. I., and Ivy, A. C.: Failure of Enterogastrone to Prevent Rumenal Ulcers in the Shay Rat, Am. J. Physiol. **148**: 382, 1947.
18. Quigley, J. P., Zettelman, H. J., and Ivy, A. C.: Analysis of the Factors Involved in Gastric Motor Inhibition by Fats, Am. J. Physiol. **108**: 643-651, 1934.
19. Shay, H., Sun, D. C. H., and Gruenstein, M.: A Quantitative Method for Measuring Spontaneous Gastric Secretion in the Rat, Gastroenterology **26**: 906, 1954.
20. Sokolov, A.: Quoted by Bobkin, B. P.: Die Aussere Sekretion der Verdauungsdrüsen, ed. 2, Berlin, 1928, Julius Springer, pp. 284-285.

JUVENILE POLYPS OF THE COLON

A Clinicopathologic Analysis of 75 Polyps in 43 Patients

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(From the Surgical Service and Pathology Department, St. Luke's Hospital)

THE commonest type of colonic polyp occurring in infants and children is the juvenile polyp. It is a pathologic entity easily differentiated histologically from other polypoid lesions of the colon. A careful review of the literature and our recent survey of over 100 surgeons and pathologists indicate considerable disagreement regarding the possibility of the development of carcinoma in the juvenile polyp. For this reason it is understandable that therapy for these lesions ranges between conservative management and radical surgery. This presentation is based on a review of the literature, the survey just mentioned, and an experience in the study and therapy of 75 polyps in 43 patients under 20 years of age. In an experience covering a 20-year period, our efforts have led us to conclude that the juvenile polyp does not possess any malignancy potential and consequently, under the proper circumstances, radical surgery is not indicated as therapy.

PATHOLOGY

Each polyp in this series was studied microscopically subsequent to either operative removal or spontaneous extrusion immediately prior to admission. Without exception the 75 polyps reviewed presented a uniform histologic appearance. Grossly the lesions are round or oval (Fig. 1). They have a smooth, continuous surface, in contrast to the finely papillary surface that characterizes the adenomatous polyp (Fig. 2). There is a slender stalk covered with colonic mucosa which is continuous with the adjacent colon mucosa. As it extends onto the bulbous surface of the polyp the epithelium is replaced by granulation tissue (Fig. 3). The bulbous portion is composed of cellular, vascular, fibrous tissue heavily infiltrated with acute and chronic inflammatory cells. In this tissue are characteristic spaces, varying in size and lined by mature, columnar, mucus-secreting epithelial cells (Fig. 4). In the stalk are fibrous tissue and strands of muscularis mucosa which fan out into the bulbous portion. Nowhere is there evidence of atypicality of cells or loss of differentiation of the epithelial elements.

Inflammatory polyps, frequently seen secondary to ulcerative colitis, do not contain these epithelium-lined spaces (Fig. 5). There is no difficulty in distinguishing this lesion from the adenomatous polyp and the papillary adenoma. The adenomatous polyp is characterized by its arborization and frequent cellular atypia (Fig. 6). The papillary adenoma is readily differentiated by its villiform, frond-like configuration (Fig. 7).

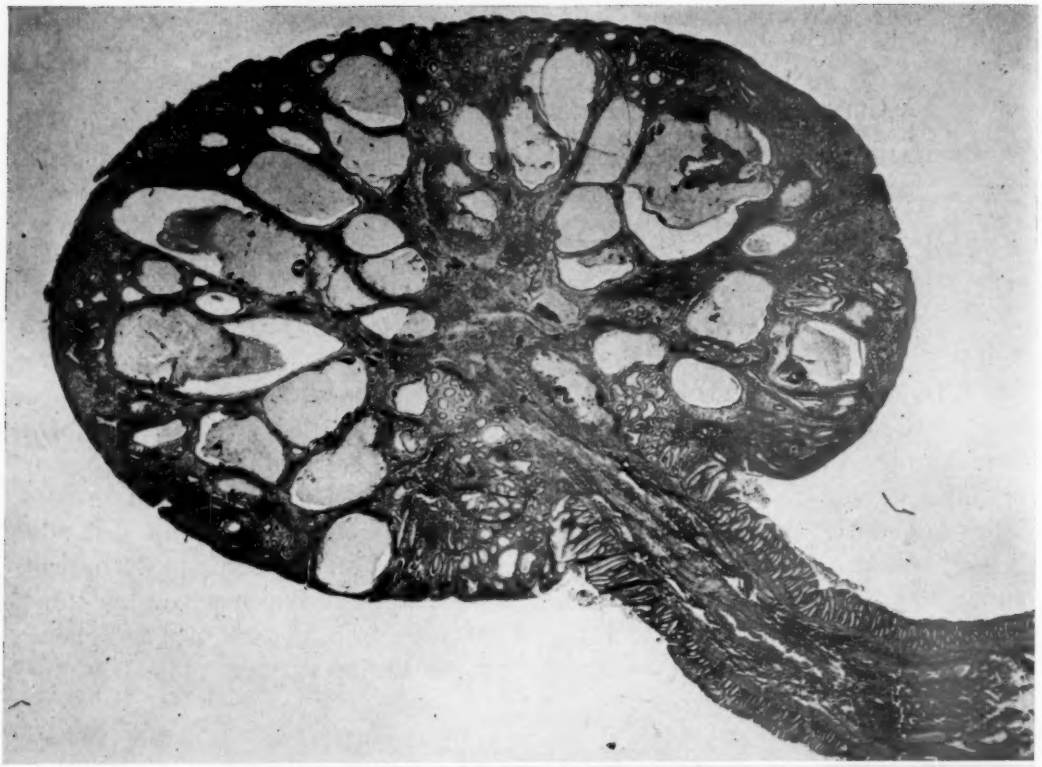
No definite cause has been found for these lesions. Infections,[9] mucosal arrests trapped in cicatrix,[13] and congenital causes[9, 22] have been suggested but have not been proved.

SURGICAL EXPERIENCE

The 43 patients comprising this report were those admitted with juvenile polyps to St. Luke's Hospital, New York City, from Jan. 1, 1939, to Dec. 31, 1959. Following a

Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

Fig. 1.



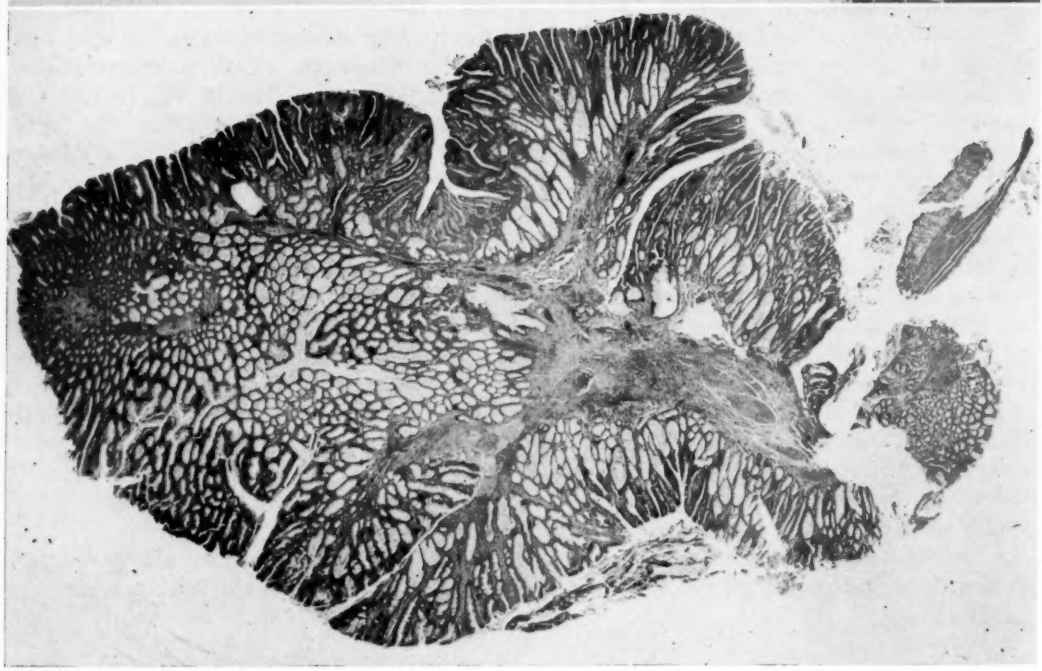


Fig. 2.

Fig. 1.—Juvenile polyp. Note continuous surface, cystic spaces. (Hematoxylin and eosin; original magnification ×8.)

Fig. 2.—Adenomatous polyp. The surface is finely papillary and the stroma is arborescent, delicate and not abundant. (Hematoxylin and eosin; original magnification ×10.)

Fig. 3.

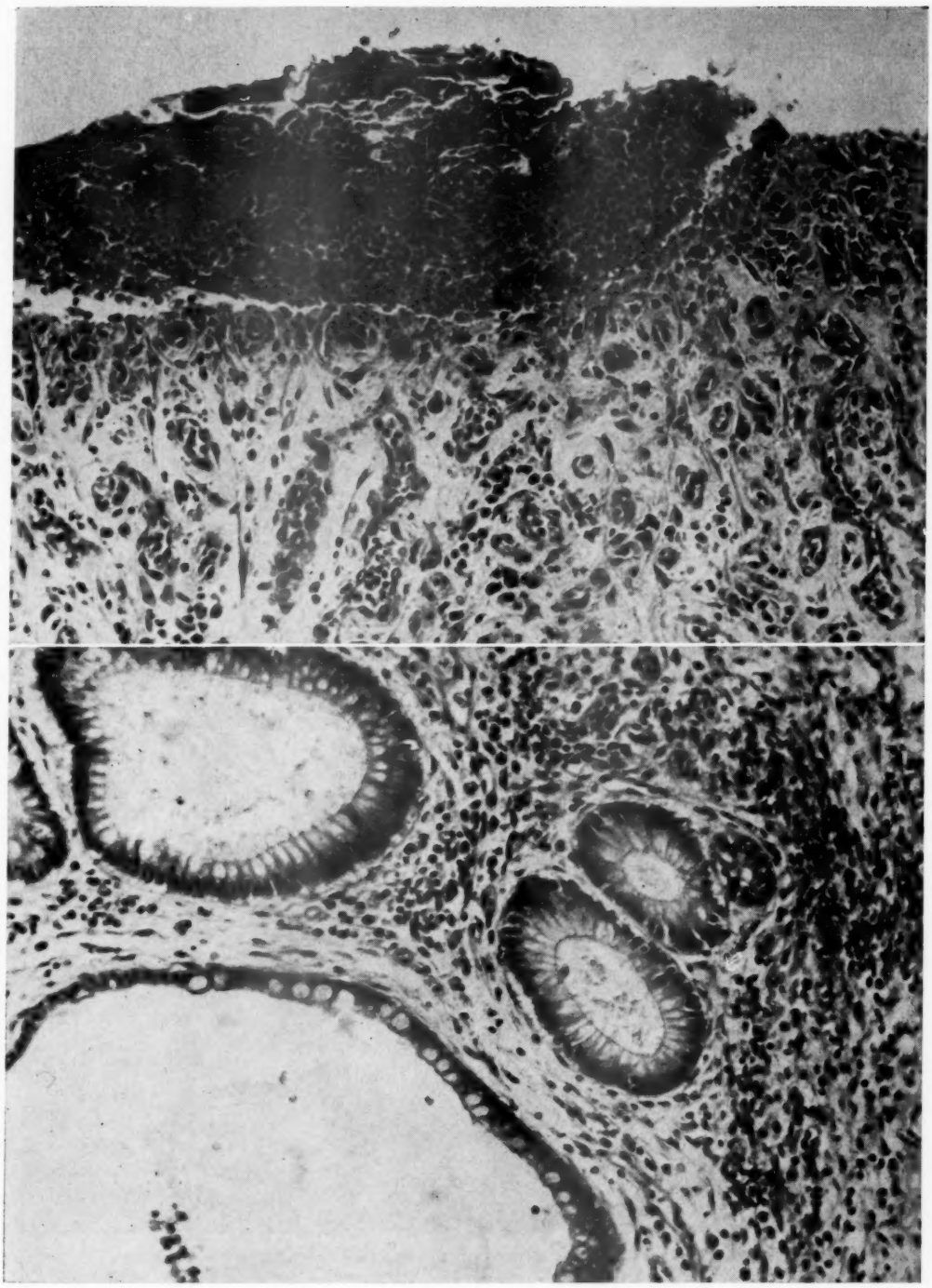
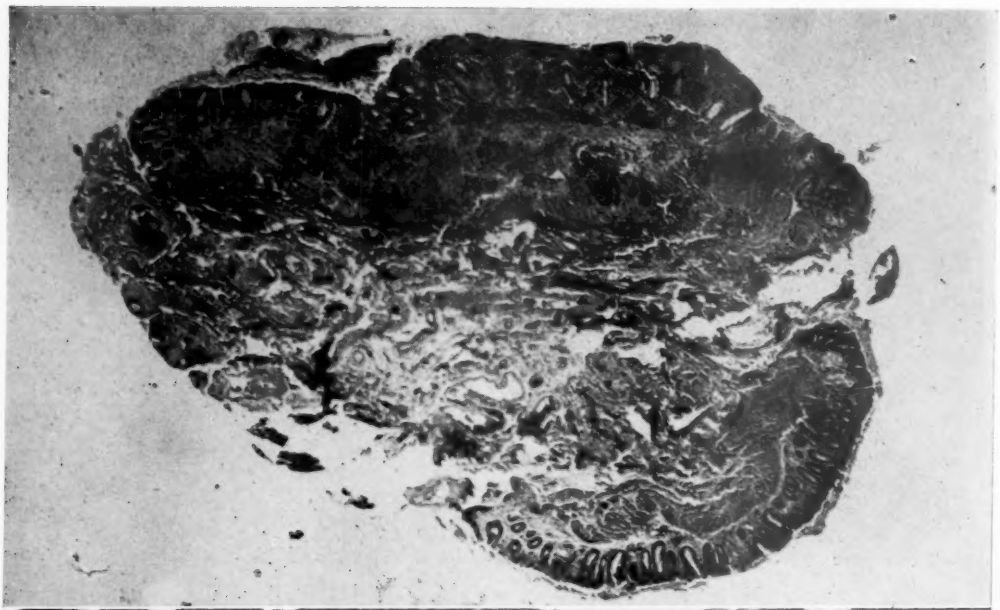


Fig. 4.

Fig. 3.—Juvenile polyp. Ulcerated surface. There are many polymorphonuclear leukocytes and eosinophils in the stroma. (Hematoxylin and eosin; original magnification ×125.)

Fig. 4.—Juvenile polyp. Cystic spaces are lined by uniform, mucus forming columnar cells. Stroma is infiltrated with inflammatory cells. (Hematoxylin and eosin; original magnification ×125.)

Fig. 5.



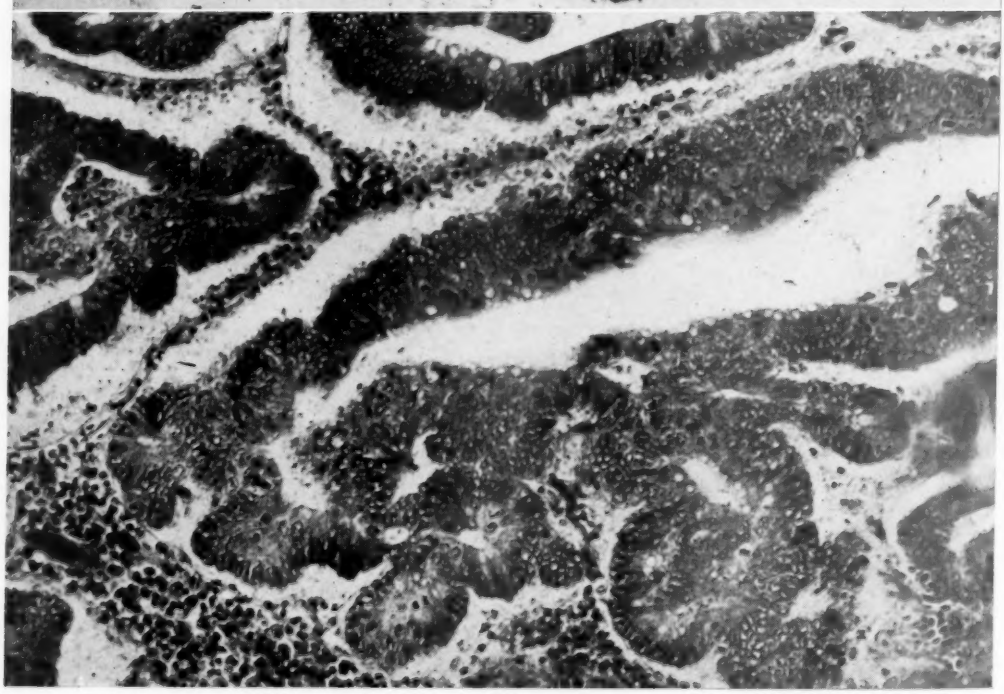


Fig. 6.

Fig. 5.—Inflammatory polyp. Abundant fibrous stroma without cystic spaces. Stroma is infiltrated with chronic inflammatory cells. Normal mucosa covers surface but may become ulcerated. (Hematoxylin and eosin; original magnification ×22.)

Fig. 6.—Adenomatous polyp. Cells covering stromal fronds are heaped up and atypical. Occasional mitroses are present and there is occasionally loss of polarity. (Hematoxylin and eosin; original magnification ×125.)

preliminary study and review of the histologic sections of 533 colonic polyps removed from patients of all ages in our hospital in the 10-year period from 1949 to 1959, it was apparent that (1) no patient with juvenile polyps was more than 20 years of age, and (2) the incidence of juvenile polyps in our experience was 5.1 per cent (Table I). We therefore, for

TABLE I. POLYPS OF COLON BY TYPE (ST. LUKE'S HOSPITAL, 1949-1958)

TYPE	NUMBER
Adenomatous	394
Inflammatory or fibrous	83
Juvenile	27
Papillary adenoma	8
Lymphoid	9
Carcinoid	8
Polyposis	4
Total	533

Fig. 7.—Papillary adenoma. This lesion is sessile and composed of delicate, branching fronds covered with somewhat atypical epithelium. (Hematoxylin and eosin; original magnification ×6.)

the main portion of this study, considered only those patients up to and including 20 years of age. No adenomatous polyps or polyps of the familial type, and only 1 case of an inflammatory polyp secondary to ulcerative colitis, were found in the patients in this age category. The largest number of juvenile polyps noted in any patient was 9. No patient in the series qualified for the diagnosis of "scattered polyposis" as defined by Gross[7] and described in detail by Gordon and associates.[6] Ages of the patients ranged from 22 months to 20 years, with a mean age of 6½ years and a median age of 5 years. Eighty per cent of the patients were less than 10 years of age with a distinct decline in incidence noted in the second decade (Fig. 8). There were 27 male patients (62 per cent) and 16 females (38 per cent). Three patients were of the Negro race.

SYMPTOMATOLOGY

The symptoms of juvenile polyps usually are the result of 3 factors: (1) the mass, per se, which may lead either to the appearance of the lesion at the anal orifice, colonic intussusception, or rectal mucosal prolapse, (2) circulatory embarrassment secondary to torsion of the pedicle, giving rise to bleeding, autoamputation, and the spontaneous extrusion of the polyp in the stool, or (3) inflammation. Rectal prolapse, stressed as a frequent accompanying symptom by Church and Schwartz,[3] Gordon and his group,[6] and Neuhof[17] was not observed in our patients. No patients in our series had intussusception or gave a history suggestive of this complaint. Indeed, the risk of this complication is rare, since only 3 patients with intussusception associated with juvenile polyps are recorded in the literature.[7] Presentation of the mass at the anus occurred in 17 (39 per cent) of the 43 patients. Circulatory embarrassment leading to tissue necrosis or autoamputation was documented in 4 (9 per cent) of the patients in the series. Inflammation, presumably the result of trauma from the fecal stream or bacterial infection, was prominent in all specimens obtained from the 43 patients. Inflammation probably accounts for the high incidence of bleeding which was the presenting symptom in 41 (95 per cent) of the patients. The bleeding was minimal in our patients, as attested by the fact that 85 per cent of the patients had admission hemoglobin levels of 11 Gm. per cent or higher and only 2 patients had levels of

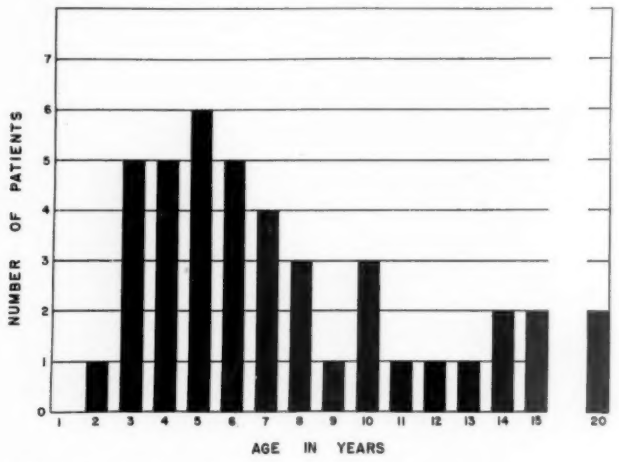


Fig. 8.—Age incidence of patients with juvenile polyps.

9 Gm. per cent. This is more interesting because 53 per cent of the patients gave a history of intermittent gross bleeding in the stool for 1 month to 1 year prior to hospitalization, and 13 per cent of the patients bled at intervals for over 1 year. Occult blood was found on stool examination in 4 (17 per cent) of 24 patients without a restricted diet, subjected to stool guaiac studies. Only 1 patient required a transfusion during hospitalization and this was necessary only subsequent to laparotomy and colotomy. Abdominal pain, diarrhea, and weakness were noted as accompanying symptoms in the history of 8 patients (19 per cent).

DIAGNOSIS

As outlined in Fig. 9, 65 per cent of the polyps were located in the rectum or rectosigmoid with a lesion palpated by digital examination in 19 (44 per cent) of the 43 patients. A combination of digital and endoscopic examination revealed the lesion in 71 per cent of the cases. A review of the current literature[3, 6-8, 10, 13, 16, 20, 23] reveals that others have had a similar experience. A double-contrast barium polyp study should be included in the work-up of all of these individuals. In this series, 13 proximally located polyps, all accompanying distal and accessible polyps, were revealed solely by roentgen studies. Single juvenile polyps occurred in 30 patients (70 per cent), and 13 patients (30 per cent) had

more than one polyp. The maximum number of lesions found in a single individual was 9, as has been previously mentioned. These figures coincide with other published series.[10, 11, 13] It is rare to have more than 3 or 4 juvenile polyps present at the time of diagnosis.[10, 14]

It is to be emphasized that in no patient in this series were polyps located solely in the colon beyond the range of the sigmoidoscope. All patients with multiple polyps therefore had one or more lesions available for histologic diagnosis by means of endoscopy.

DISCUSSION

As mentioned previously, there is considerable confusion among pathologists and surgeons regarding the malignancy potential of juvenile polyps. This was demonstrated by the response to our questionnaire to which 71 per cent of the authorities polled responded. This reaction was interpreted by us to indicate that there is considerable interest in attempting to determine the exact nature of these lesions and their premalignant or nonpremalignant status. Fifty-five per cent of the individuals surveyed believed that the malignancy potential of this type of lesion is not fully understood. Only 20 per cent of the replies indicated that these polyps should be considered as premalignant, yet 83 per cent advised their removal, regardless of whether the polyps were symtomatic or not, and regardless of whether or not a colotomy was necessary.

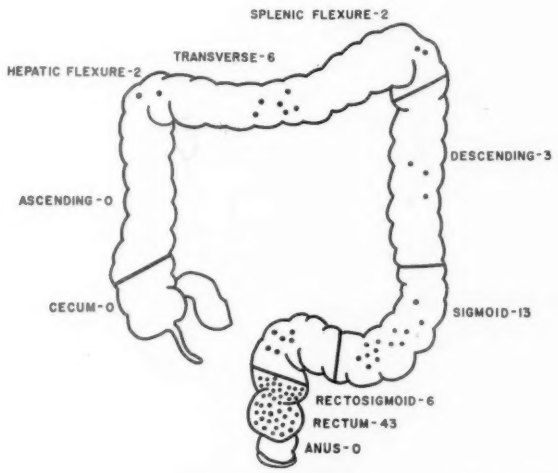


Fig. 9.—Distribution of 75 juvenile polyps in 43 children (St. Luke's Hospital, 1939 to 1959).

The lack of proper pathologic description and terminology has undoubtedly added to the confusion. Juvenile polyps have been called adenomas,[2, 4, 5, 9] papillomas,[21] low grade 1 adenocarcinomas,[12] inflammatory redundancy associated with rectal prolapse,[3, 17] and cystic or retention polyps.[1, 4, 9]

Because of our experience reported herein and the fact that a careful review of the literature failed to produce any evidence that juvenile polyps are premalignant, we believe that a conservative approach to therapy should be employed. Juvenile polyps should not be treated as precancerous lesions as are adenomatous polyps and the lesions of hereditary polyposis, both well authenticated as to malignancy potential.

It may be stated that 10 patients out of the 43 underwent a laparotomy for proximally located polyps. In 3 of these individuals bleeding polyps were

excised by means of the proctoscope, yet colotomy was performed for additional asymptomatic polyps situated in the proximal colon. Had a conservative regime been followed, these 3 patients might well have been spared an operative procedure. In addition, it is of interest to record that in 2 other patients, subjected to abdominal exploration for sigmoid polyps well documented radiographically, no polyps were located. Whether or not self-amputation had occurred in the interval between diagnosis and operation can only be presumed. One other child in this group of 10 operated upon transabdominally required a second laparotomy for lysis of adhesions 6 years subsequent to the original polypectomy.

In regard to symptomatology, Kerr[13] has reported that 18 per cent of his patients experienced spontaneous extrusion of a juvenile polyp. This contrasts with the finding of 9 per cent in our series. The documented evidence of a decrease in incidence occurring in the second decade of life lends credence to the theory that many of these lesions, if observed, will undergo torsion and self-amputation. Rectal bleeding as a presenting symptom has been adequately emphasized as a predominant symptom in the literature.[10, 13, 16]

Follow-up studies are of significance for 2 reasons. First, these polyps are known to recur in new locations in certain individuals. Turell and Maynard[23] noted this phenomenon in 2 of 68 patients (3 per cent) studied, and Horrilleno and colleagues[10] documented recurrence in 10 children of a series of 55 observed in a follow-up period ranging from 10 weeks to 10 years. In one of their patients 27 polyps of the juvenile type were removed over a period of 8 years, the number being treated at any one time totaling 7. Harris[8] reported 2 patients with recurrent polyps in a series of 12 individuals undergoing laparotomy and colotomy for juvenile polyps. In the present series, follow-up of 24 patients for periods up to 14 years revealed the appearance of juvenile polyps in a new site in 3 patients (7 per cent). None of the 24 patients developed adenocarcinoma. The second reason for the significance of follow-up studies is that if conservative management is employed for these patients with residual polyps left in the sigmoid colon, the lesions must be observed for symptoms of bleeding, pain, extrusion, and so forth. Serious bleeding, as emphasized above, has rarely been recorded.

CONCLUSION

Juvenile polyps are an easily recognized pathologic entity. According to present knowledge they are not premalignant lesions and are not known to exist in association with a definite premalignant polyp such as the adult-type adenomatous polyp. Therefore, radical surgical procedures are not required. Furthermore, in many instances autoamputation indicates that they are a self-limited disease.

Our records reveal that no patient had a juvenile polyp high in the colon unless one or more such polyps were available for removal by sigmoidoscopic examination. Thus, a histologic examination was always available without laparotomy and colotomy.

It is recommended that all polyps accessible to the sigmoidoscope should be removed and studied histologically. First, this must be done to

establish the absolute diagnosis in at least one of the polyps. Second, accessible polyps should be removed to reduce the complication of presentation of the lesion at the anus and the rare complications of rectal prolapse and intussusception.

Adenomatous polyps showing malignant change very rarely occur in infants and young adults.[13, 15, 18, 19] However, since the coexistence of juvenile polyps and adenomatous polyps has not been recorded, more extensive operative procedures should not be employed to remove asymptomatic juvenile polyps in the proximal colon.

Although the phenomenon did not occur in our series, if a patient has a polyp in the upper colon and none is found on sigmoidoscopic examination, that child must undergo a colotomy and removal of at least 1 polyp to establish the diagnosis. After excision, patients with juvenile polyps should be followed because of the possibility of recurrence. A new lesion should be removed and examined microscopically.

SUMMARY

1. A series of 75 juvenile polyps occurring in 43 patients between the ages of 22 months and 20 years is presented.

2. The majority of these lesions occur in the first decade of life and are associated with rectal bleeding of a minor nature as the most prominent symptom.

3. Juvenile polyps should be considered as a distinct entity from other polyps of the large bowel. A uniform pattern of gross and microscopic architecture was noted in each lesion in this series.

4. The malignancy potential of juvenile polyps has not been demonstrated.

5. Juvenile polyps, located in the proximal bowel not accessible to removal by the sigmoidoscope, may be observed rather than excised if asymptomatic and if a diagnosis has been established following removal of a more distally located lesion.

REFERENCES

1. Ackerman, L. V.: Surgical Pathology, St. Louis, 1953, The C. V. Mosby Company.
2. Castro, A. F.: Adenomatous Polyps of Rectum and Colon in Children, Clin. Proc. Children's Hosp. **11**: 29, 1955.
3. Church, R. E., and Schwartz, A. D.: Polyps of Rectum and Colon in Children, J. Pediat. **44**: 104, 1954.
4. David, V. C.: Some Etiological and Pathological Factors in Cancer of the Large Bowel, Arch. Surg. **41**: 257, 1940.
5. Dewis, J. W.: Small Fibroma of Ileum Resulting in Obstruction of the Bowel With Consideration of Various Forms of Benign Intestinal Tumors, Boston M. & S. J. **155**:: 427, 1906.
6. Gordon, D. L., Hallenbeck, G. A., Dockerty, M. A., Kennedy, R. L. J., and Jackman, R. J.: Polyps of the Colon in Children, A. M. A. Arch. Surg. **75**: 90, 1957.
7. Gross, R. E.: The Surgery of Infancy and Children, Philadelphia, 1953, W. B. Saunders Company.
8. Harris, J. W.: Polyps of the Rectum and Colon in Children, Am. J. Surg. **86**: 577, 1956.
9. Helwig, E. B.: Adenomas of the Large Intestine in Children, Am. J. Dis. Child. **72**: 289, 1946.
10. Horrilleno, E. G., Eckert, C., and Ackerman, L. V.: Polyps of the Rectum and Colon in Children, Cancer **10**: 1210, 1957.
11. Kennedy, R. L. J.: Polyps of the Rectum and Colon in Infants and Children, Am. J. Dis. Child. **62**: 481, 1941.

12. Kennedy, R. L. J., Dixon, C. F., and Weber, H. M.: Polypoid Lesions of the Colon in Children, Surg. Gynec. & Obst. 77: 639, 1943.
13. Kerr, J. C.: Polyposis of the Colon in Children, Am. J. Surg. 76: 667, 1948.
14. Ladd, W. E., and Gross, R. E.: Abdominal Surgery of Infancy and Childhood, Philadelphia, 1941, W. B. Saunders Company.
15. Marion, J., and Avet, J.: Malignant Degeneration of Rectal Polyp in Child of Four Years, Rev. Mens de Pediatrie, Lyons, 584, 1948.
16. Mauro, J., and Prior, J. T.: Gastrointestinal Polypoid Lesions in Childhood, Cancer 10: 131, 1957.
17. Neuhof, H.: Rectal Polyp in Childhood, New York J. Med. 101: 1056, 1915.
18. Osting, M.: Adenocarcinoma of Sigmoid Colon, Rectum and Anus in Children. Report of Two Cases in 13-year-old Girl and 8-year-old Boy With Summary of Recorded Cases up to 15 Years of Age, Ohio M. J. 37: 1067, 1941.
19. Schilla, W. W.: Carcinoma in a Rectal Polyp (Report of a Case in Infancy), Am. J. Surg. 88: 759, 1954.
20. Snyder, W. H., Jr., Chaffin, L., and Snyder, M. H.: Neoplasms of the Colon and Rectum in Infants and Children. A Survey of Polyps, Polyposis and the Uncommon Malignant Lesions of the Large Bowel, Symposium on Pediatric Proctology, Pediat. Clin. North America 3: 93-111, 1956.
21. Sunderland, D. A., and Buckley, G. F.: Papillary Adenomas of the Large Intestine: Clinical and Morphological Study of 48 Cases, Cancer 1: 184, 1948.
22. Swinton, N. W., and Warren, S.: Polyps of the Colon and Rectum and Their Relation to Malignancy, J. A. M. A. 113: 1927, 1939.
23. Turell, R., and Maynard, A. DeL.: Adenomas of Rectum and Colon in Juvenile Patients, J. A. M. A. 161: 57, 1956.

Discussion

DR. C. EVERETT KOOP (Philadelphia, Pa.).—In the 15 years that I have confined my surgical endeavors to the problems of childhood, we have seen but 4 adenomatous polyps. One was in a 14-year-old boy, who had intussusception of the sigmoid colon. Three of these were in the same child. All 4 of these polyps seen on barium enema indicated that they were not the usual type of juvenile polyp described here. I could agree with practically everything that has been said here about juvenile polyps. Bleeding has never been a hemorrhage in our experience. It has seldom produced anemia. So many patients pass their polyps while waiting for operation that I cannot help but believe that this is part of the natural life history of this peculiar lesion. In the several years that I was on the tumor registry of the American Academy of Pediatriacs, there was no single instance to my knowledge where a carcinoma of the bowel developed in a polypoid lesion. As a matter of fact, statistics are very much in favor of the surgeon who treats this type of lesion in childhood because, as you know, less than 6 per cent of any childhood cancer is epithelial. I think one question might be raised and that is: when does one stop thinking about the patient with a juvenile polyp as though he were a child and begin thinking of him more in terms of adult surgical experience? Inasmuch as pediatric cancer asymptotically approaches zero in a person at the age of 17, this might be the magic age where one shifts and begins treating lesions as they are treated in adults.

DR. ECKERT (closing).—The authors are to be congratulated for their very complete study of these interesting lesions. It is disappointing to learn the results of this poll, for in 1957 we reported a similar study with identical conclusions. Of greatest importance is the fact that cancer of the colon or rectum has not occurred in the follow-up of patients with these lesions. I would like to illustrate an example of overtreatment of juvenile polyps. This slide shows the colon from 1 of 2 siblings with indentical lesions. The diagnosis had been made in both by a biopsy of a rectal polyp. The next slide show one of these polyps which, as you will see, is typical of the lesions illustrated by the authors. Today we would certainly not perform a colectomy for multiple juvenile polyps. Undoubtedly there are many other patients who are overtreated because of failure to distinguish between juvenile and adult polyps.

APPLICATION OF THE ELECTROMAGNETIC FLOWMETER TO VASCULAR STUDIES IN HUMAN PATIENTS

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THE measurement of volume blood flow through various vessels and organs has innumerable applications in experimental as well as clinical conditions. Most methods of direct measurement have not been applicable to patient studies since they require division or cannulation of the vessel to be studied. This paper reports on early experience with the use of the electromagnetic flowmeter for vascular studies in patients.

The basic physical principle involved in this flow-measuring device is that the movement of an electrical conductor through a magnetic field produces a voltage across the conductor which is proportional to the number of magnetic lines of force cut per unit time. A stream of blood moving through a vessel is a conductor of electricity. If a magnetic field is supplied about this moving conductor by an external magnet device, a voltage is induced across the vessel which can be picked up by suitable electrodes and calibrated in terms of flow. The square-wave electromagnetic flowmeter† of Denison and Spencer[1] was used in the present study. A magnet-assembly sensing device, usually referred to as a probe, is shown in Fig. 1. The probe can be sterilized in one of the cold sterilizing solutions, then attached to an extension cord of any convenient length. It is used by slipping the open end of the probe over the exposed but intact vessel. A variety of probe sizes must be available to enable accurate coaptation of probe to vessel wall. Calibration is accomplished by passing blood at precisely measured rates through excised vessels to which the probe is applied. At the present time, zero flow must be determined by momentary occlusion of the vessel, but there is reason to believe that in the future the zero point may be obtained electronically.

Blood flow has been measured in the operating room during a variety of procedures. Several examples of such measurements are presented here to illustrate the type of information which can be obtained in this manner.

RESULTS

Flow studies were performed during the course of a saphenous vein ligation on a 62-year-old white man without evident arterial disease. Instantaneous flow patterns from the superficial femoral artery are shown in Fig. 2.

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*Buswell Fellow in Surgical Research.

†Built by Carolina Medical Electronics, Winston-Salem, N. C.

In the normal femoral pattern seen on the left, attention is called to the position of zero flow. From one-fourth to one-third of the flow was below the zero flow line and, hence, represents reverse flow. On the right is shown the effect of 50 mg. of Priscoline given intra-arterially; all back flow ceased and total flow amplitude increased.

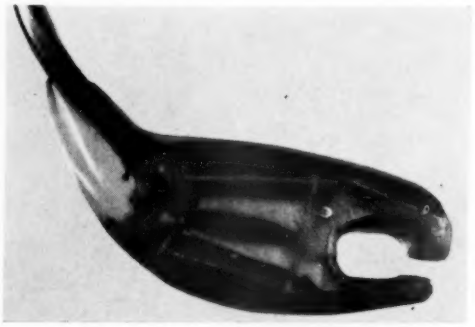


Fig. 1.—Electromagnetic flowmeter probe. "U" magnet with electrical windings can be seen through encasing plastic. Vessel fits in C-shaped slot.

Fig. 2.

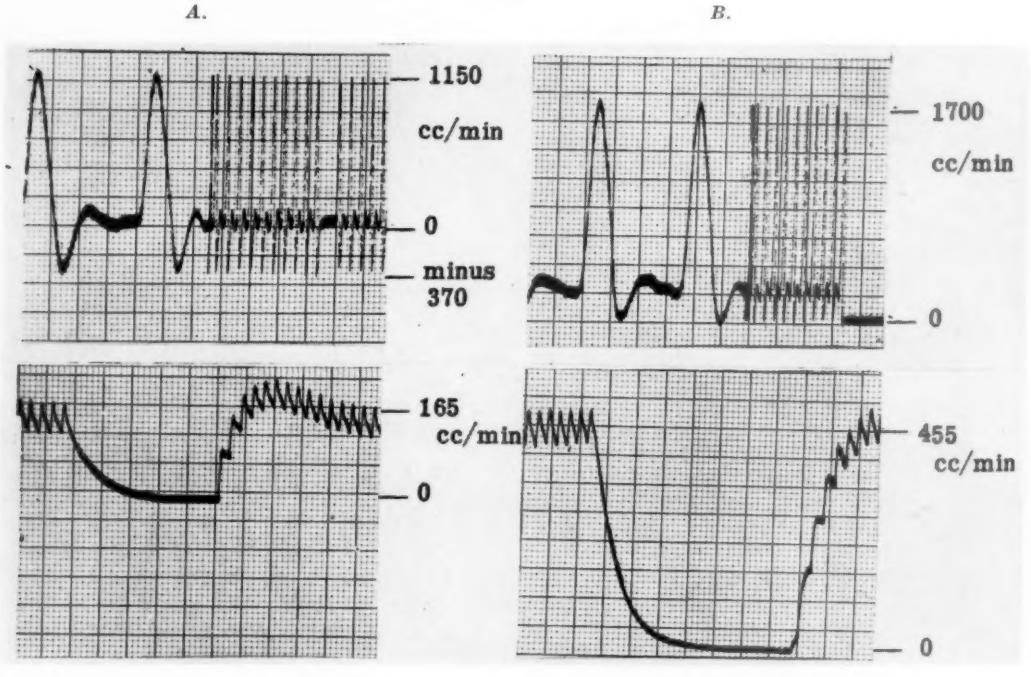


Fig. 3.

Fig. 2.—Instantaneous left superficial femoral artery pulsatile flow recordings before *(A)* and after *(B)* intra-arterial Priscoline injection.

Fig. 3.—Mean left superficial femoral artery mean flow recordings before *(A)* and after *(B)* intra-arterial Priscoline injection.

The method of determining mean flow is shown in Fig. 3. Electrical damping is introduced into the flow-recording circuit so as to obtain a geometric mean figure. For illustrative purposes, flow tracings are shown in Fig. 3 which are not completely damped. The zero flow point seen on these curves

was obtained by momentary occlusion of the vessel downstream from the flow probe. The mean flow alteration produced by Priscoline in this patient is shown in Table I. A 2.5- to 3.0-fold increment in flow through this vessel occurred with little change in arterial pressure.

TABLE I. MEAN FLOW IN SUPERFICAL FEMORAL ARTERY BEFORE AND AFTER INTRA-ARTERIAL INJECTION OF PRISCOLONE IN A 62-YEAR-OLD WHITE MAN WITHOUT EVIDENT ARTERIAL DISEASE (C.C./MIN.)

	CONTROL FLOW	FLOW 12 MINUTES AFTER INJECTION OF 50 MG. PRISCOLONE INTRA-ARTERIALLY
Left superficial femoral artery	168	455
Right superficial femoral artery	144	445
Blood pressure (left arm)	160/90	160/100

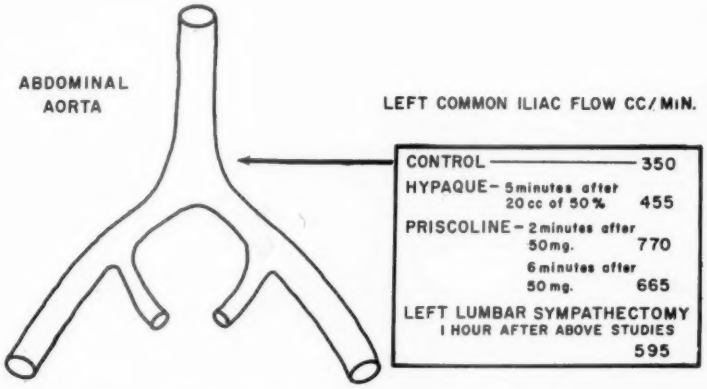

ABDOMINAL AORTA
LEFT COMMON ILIAC FLOW CC/MIN.
CONTROL 350
HYPAQUE - 5 minutes after 20 cc of 50% 455
PRISCOLINE - 2 minutes after 50 mg. 770
6 minutes after 50 mg. 665
LEFT LUMBAR SYMPATHECTOMY
1 HOUR AFTER ABOVE STUDIES
595

Fig. 4.—Common iliac artery mean flow determinations, in a 71-year-old man with arterial insufficiency, as influenced by intra-arterial contrast material and vasodilator and lumbar sympathectomy.

We have frequently made the clinical observation that patients with arterial insufficiency seem to obtain temporary relief of symptoms after arteriography. Flow studies were performed on the left common iliac artery during the course of a lumbar sympathectomy as shown in Fig. 4. A flow increment of 100 c.c. per minute followed Hypaque injection. Subsequent Priscoline injection resulted in flows which were twice the control level. The change after sympathectomy is difficult to interpret because of the probable residual effects of the earlier pharmacologic agents. The effect of lumbar sympathectomy is shown in Figs. 5 and 6. Control flow shown in Fig. 5 in this instance approximately doubled after lumbar sympathectomy as seen in Fig. 6. The characteristics of this flow alteration are seen in Fig. 7, which shows the flow-wave forms in this vessel before and after sympathectomy. A pronounced shift in the zero reference followed sympathectomy, indicating a significant alteration in peripheral resistance.

Further studies of the immediate results of denervation were obtained during transperitoneal bilateral lumbar sympathectomy. Results from one

such study are summarized in Table II. Flow through the left common iliac artery was doubled by sympathetic denervation on the left side with no significant accompanying change on the right. Subsequent denervation on the right produced a small decrement in flow on the right with no change on the left.

In another similar case it was possible to make flow studies only on the right common iliac artery during the course of bilateral sympathectomy. Table III shows these results. Flow through the right common iliac artery increased rather strikingly following ipsilateral sympathectomy, but returned to approximately the control value when the contralateral side was denervated.

TABLE II. ALTERATION IN FLOW THROUGH COMMON ILIAC ARTERIES FOLLOWING UNILATERAL AND BILATERAL LUMBAR SYMPATHECTOMY IN A 61-YEAR-OLD MAN WITH ATHEROSCLEROTIC ARTERIAL INSUFFICIENCY

	PRESYMPATHECTOMY	AFTER LEFT LUMBAR SYMPATHECTOMY	AFTER BILATERAL LUMBAR SYMPATHECTOMY
Right common iliac artery	350	375	285
Left common iliac artery	315	700	700
Total flow through both common iliacs	665	1,075	985
Arterial blood pressure	120/80	117/78	110/70

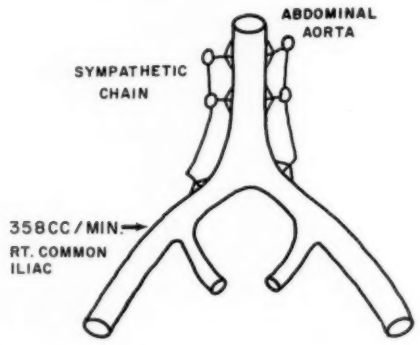


Fig. 5.

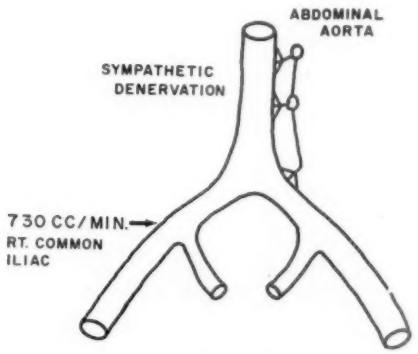


Fig. 6.

Fig. 5.—Right common iliac artery mean flow determinations before lumbar sympathectomy in a 71-year-old man with intermittent claudication and thermal burns of toes.

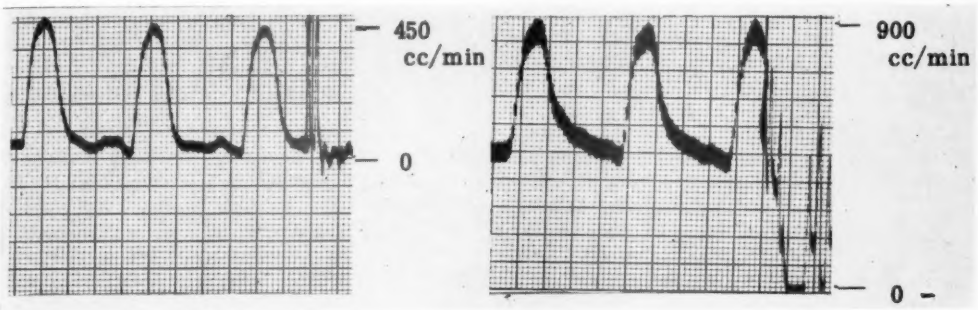
Fig. 6.—Right common iliac artery mean flow determinations immediately after lumbar sympathectomy in same patient as in Fig. 5.

A final illustration of lumbar sympathectomy effect is shown in Table IV. Flow through the right common iliac artery doubled following sympathetic denervation but this flow increment was accompanied by a corresponding decrement of flow through the opposite iliac artery, so that the total flow through the 2 limbs remained unchanged.

It has been of considerable interest to attempt the quantitation of flow alteration resulting from vascular reconstruction for atherosclerotic occlusive disease. Volume flow through both external iliac vessels in a patient with the Leriche syndrome is shown in Fig. 8. This very small flow resulted entirely from the retrograde flow from collateral vessels through the internal iliac arteries. Following prosthetic bypass reconstruction, flow recordings were as

TABLE III. MEAN FLOW THROUGH RIGHT COMMON ILIAC ARTERY FOLLOWING IPSILATERAL AND BILATERAL LUMBAR SYMPATHETIC DENERVATION IN A 52-YEAR-OLD WHITE MAN WITH MARKED ARTERIAL INSUFFICIENCY AND SCLEROTIC ILIAC VESSELS

	RIGHT COMMON ILIAC ARTERY FLOW (C.C./MIN.)
Control	235
Right lumbar sympathectomy	750
Bilateral lumbar sympathectomy	280



A. *B.*

Fig. 7.—Instantaneous flow recordings from right common iliac artery before *(A)* and after *(B)* lumbar sympathectomy.

TABLE IV. ALTERATION IN DISTRIBUTION OF MEAN BLOOD FLOW THROUGH COMMON ILIAC ARTERIES BEFORE AND AFTER RIGHT LUMBAR SYMPATHECTOMY IN A 60-YEAR-OLD NEGRO MAN DIABETIC WITH ISCHEMIC CHANGES OF RIGHT SECOND TOE (C.C./MIN.)

	BEFORE RIGHT LUMBAR SYMPATHECTOMY	AFTER RIGHT LUMBAR SYMPATHECTOMY
Right common iliac artery	260	540
Left common iliac artery	600	320
Total flow through both common iliacs	860	860

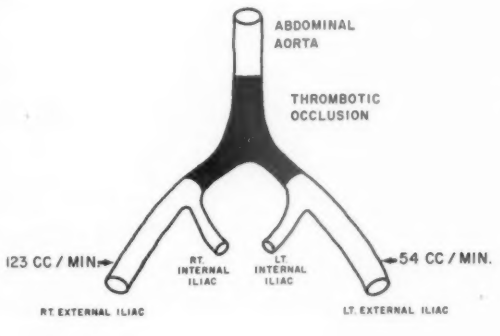


Fig. 8.

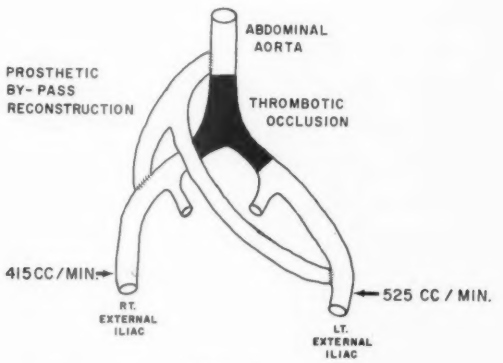


Fig. 9.

Fig. 8.—Mean flow values through external iliac arteries in 51-year-old patient with Leriche syndrome with abdominal aortic occlusion.

Fig. 9.—Mean flow values through external iliac arteries after bypass reconstruction of abdominal aortic occlusion in same patient as in Fig. 8.

seen in Fig. 9. It should be pointed out that even these greatly increased flows do not represent the entire flow through the limbs of the prosthesis. There is little doubt that both antegrade and retrograde flow occurred in the external iliac artery from the site of the end-to-side anastomoses. In this way a normal direction of flow in the internal iliac arteries was presumably restored. Measurement of this retrograde flow could not be accomplished without additional dissection.

During the course of the repair of a coarctation of the aorta in a 16-year-old white boy, it was possible to make flow determinations on the descending aorta just proximal to the coarctation and on the left subclavian artery. The results of this study are seen in Table V. Immediately after excision and

TABLE V. MEAN BLOOD FLOW THROUGH DESCENDING THORACIC AORTA AND LEFT SUBCLAVIAN ARTERY BEFORE AND AFTER EXCISION AND REPAIR OF COARTATION OF AORTA IN A 16-YEAR-OLD WHITE BOY (C.C./MIN.)

	PRIOR TO REPAIR	AFTER REPAIR
Descending aorta	780	1,550
Left subclavian artery	310	125
Brachial blood pressure	170/70	140/70

Fig. 10.—Instantaneous flow patterns from popliteal artery in patient with arteriovenous fistula

repair, the descending aorta flow doubled while that in the left subclavian artery was reduced by more than half. The descending aorta just proximal to the coarctation had an internal diameter of 14 mm., whereas the narrowest point of the constriction was 4 mm. Hence, 780 c.c. per minute continued to flow through a constriction which represented a 92 per cent reduction in aortic cross-section area.

Flow studies were also obtained from the popliteal artery and vein proximal to a traumatic arteriovenous fistula. The pulsatile wave form in the popliteal artery is shown in Fig. 10. The striking difference between this recording and the normal pattern may be gained by comparison with the control tracings of Fig. 2. Rather than exhibition of backflow during a portion of the cycle during diastole, there was continuous forward flow. Mean flows through the popliteal artery and vein were 1,080 and 1,500 c.c. per minute, respectively, in a 40-year-old white man. Both of these values exceed the highest flow we have yet recorded in a common iliac artery and the popliteal vein flow is equaled only by the flow through the descending aorta following

coarctation repair. The popliteal vein flow is equal to one-fourth of the predicted normal cardiac output in this patient.

On 3 occasions we have had the opportunity to make direct measurements of hepatic blood flow during the course of cholecystectomy for gallstones in patients without evident hepatic disease. In 2 of the 3 patients, it was necessary to make individual flow measurements on the right and left hepatic arteries because of a very proximal division of that vessel. The results of these studies are seen in Table VI. Although all 3 patients were of approximately the same

TABLE VI. HUMAN HEPATIC BLOOD FLOW STUDIES CARRIED OUT AT LAPAROTOMY (C.C./MIN.)

PATIENT	PORTAL VEIN FLOW	RIGHT HEPATIC ARTERY FLOW	LEFT HEPATIC ARTERY FLOW	TOTAL HEPATIC ARTERY FLOW	TOTAL LIVER BLOOD FLOW	RATIO PORTAL VEIN : HEPATIC ARTERY
E. B.	810	63	50	113	923	7.2:1
H. S.	1,100	---	---	510	1,610	2.2:1
C. S.	500	80	135	215	715	2.3:1

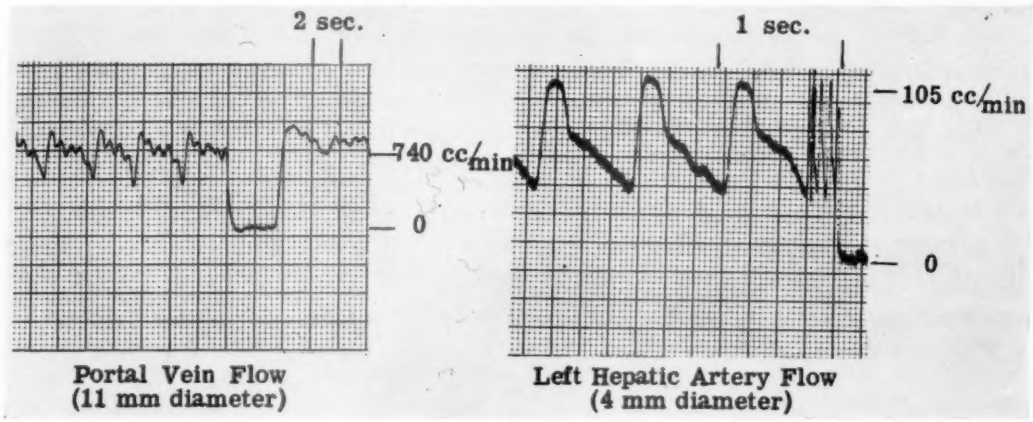


Fig. 11.—Instantaneous flow recordings from portal vein and hepatic artery in normal human patient.

body weight, there was a wide variation in total hepatic flow. In addition, the portal vein to hepatic artery flow ratio was very close in 2 studies but widely divergent in the third. Pulsatile flow patterns are shown in Fig. 11. The small, positive peaks in the portal vein recording are synchronous with cardiac systole while the larger, negative peaks are synchronous with inspiration. There is no reverse flow component to the hepatic artery flow pattern.

DISCUSSION

Extreme caution must be used in attempting interpretation of these flow studies. They were carried out in patients under anesthesia during operative procedures and often during the use of mechanical ventilators. The flow changes resulting from these surgical procedures or drugs represent the immediate effects only and may be markedly changed with passage of time. In spite of these obvious problems, it would seem that useful information may result from such investigations.

We must make flow determinations on all vessels of suitable size which are exposed during the course of operative procedures on patients free of vascular disease. Only in this way will we be able to assemble information regarding the range of alteration in instantaneous flow wave form and of alteration in mean flow which can be regarded as normal in a given vessel. This information about normal flows is obviously essential to intelligent interpretation of the abnormal flow states.

Only a series of observations of statistically valid size can enable us to predict what is most likely to happen after a given surgical or experimental procedure, but even a single careful study provides much new and useful information. With the afore-mentioned limitations in mind, the observations made may be summarized as follows: (1) Arterial blood flow increased strikingly in both normal and sclerotic vessels following intra-arterial injections of Priscoline. (2) Intra-arterial Hypaque as a contrast medium produced a temporary increase in arterial flow. (3) Lumbar sympathectomy produced a significant increment in the ipsilateral common iliac artery flow. On one occasion this was accompanied by a concomitant decrement in the contralateral iliac artery flow. The ipsilateral increment in common iliac flow which followed lumbar sympathectomy in another case was abolished by sympathectomy on the opposite side. (4) Vascular reconstruction resulted in an enormous increase in flow through the distal patent channels. (5) Relatively high flow continued through an aortic coarctation which represented a 92 per cent reduction in cross-section area. (6) One fourth the expected cardiac output was found to be flowing through the proximal popliteal vein of a traumatic arteriovenous fistula. (7) Portal vein and hepatic artery flow studies showed rather wide variation in total hepatic blood flow and in the artery-to-vein flow ratios in patients with apparently normal livers.

CONCLUSIONS

1. The square-wave electromagnetic flowmeter can be safely used for recording of both instantaneous and mean flow through surgically exposed but unopened blood vessels.

2. Alterations in flow are demonstrated before and after intra-arterial vasodilators, lumbar sympathectomy, and vascular reconstructions. Portal vein and hepatic artery flow were recorded at laparotomy.

REFERENCE

1. Denison, A. B., Jr., and Spencer, M. P.: Square-Wave Electromagnetic Flowmeter Design, Rev. Scientific Instr. 27: 707, 1956.

DISCUSSION

DR. GEORGE H. A. CLOWES, JR. (Cleveland, Ohio).—I think this is a most interesting and ingenious study. Dr. Schenk mentioned that these tests were performed under anesthesia. Before we go into vascular physiologic phenomena, all of us must be aware of what anesthesia does to the circulation. We are very impressed with this in our own studies of cardiac output. It is not at all unusual to see a man's circulation drop to one-third of

his normal basal output during deep anesthesia, particularly with spinal anesthetics or when the chest is opened. If the flow in one leg goes down when the other is denervated, this may be caused by the anesthetic. This may not be true in the intact conscious organism which is capable of increasing the venous return and cardiac output in response to a reduction of the peripheral resistance in any one vascular bed. There are reservoirs that squeeze blood into the circulation, putting up the return of venous blood to the heart. Sjostrand, in the *Physiological Reviews* in 1953, presents a very good case for the rapid increase of cardiac output by reduction of the volume of blood in the pulmonary circulation to augment the venous return to the left heart. Physiologists have denied this for years simply on the basis that they did their work under anesthesia with an open chest. Therefore, I think that observations made during operations, intriguing as they are, must be considered in this light until checked in the conscious patient by this or other means such as plethysmography.

RICHARD M. PETERS (Chapel Hill, N. C.).—We have had some experience with the flowmeter Dr. Schenk has used and also with a model which is not quite as satisfactory made by a group in California. While we have not started to use it clinically in patients, we have found it very useful in the laboratory. Our interest has centered on the effects of portal vein occlusion on the cardiac output. Occlusion of the portal vein leads to a progressive fall in cardiac output associated with a fall in systemic pressure. These changes are out of all proportion to the fall in cardiac output that occurs following occlusion of the inferior vena cava above the renal veins. These findings lend support to the idea that the portal circulation may be a storage area for blood. We have carried out one experiment in which we have tried to use 2 flowmeters simultaneously, one to determine the flow in the inferior vena cava and the other the flow in the ascending aorta. This represents new hazards which Dr. Schenk helped me to clarify—that one magnetic probe does begin to have influence on the other. Ultimately, the evaluation of flow distribution may be important in extracorporeal circulation, hypothermia, and shock to determine where the limited amount of blood is going.

DR. RALPH A. DETERLING, JR. (Boston, Mass.).—I think instruments like this are extremely important in developing our understanding of human physiology. There appears to be some conflict in the paper just presented with fairly well-established figures which have been repeatedly confirmed by measurements with the thermostromuhr of Rehn and more modern devices. As I recall, such studies indicate that approximately 50 per cent blood flow will result with a constriction or reduction of cross-sectional area of about 70 per cent. I wonder if, in the coaretation case, where only a 50 per cent reduction was reported with an estimated 92 per cent constriction, we were not actually seeing a measurement of the return of blood flow into the thoracic aorta, by the intercostal arteries distal to the constriction? I would also like to know how the 92 per cent was estimated because an estimation from an excised specimen is quite different from that of one which is functioning with an elevated blood pressure.

DR. SCHENK (closing).—I wish to thank Drs. Clowes, Peters, and Deterling for their comments. They are very pertinent. We are certainly aware of the effects of anesthesia in any flow study, and that is the reason why we inserted the statement of caution in our own remarks. There is the hope that in the future it may be possible to implant probes or some type of probe device which can be removed without difficulty, so that flow measurements may be made not only in unanesthetized patients, but even in patients exercising on a treadmill. This is looking into the future. As Dr. Peters said, this is an extremely useful experimental tool in the laboratory although I must admit it is not an easy one to use, and we have had 3 years' experience in the laboratory before applying the instrument to patients. There is a field for enormous electronic improvement in the circuitry. If there would be sufficient incentive given to the electronics people, this circuit could be transistorized with enormous improvement in its performance.

With reference to the coarctation that Dr. Deterling spoke about, most of the studies in relation to change of flow with constriction have been with a fixed proximal pressure head and with coarctation, as in our study there was a significantly elevated proximal pressure head. I believe, as Dr. Wiggers has shown, that if you elevate the head of pressure proximal to a constriction, considerable flow can continue through a rather markedly narrowed orifice. Of course, our estimates of diameter can easily be criticized because we distended the excised specimen as best we could, to make a measurement of the constricted portion after excision, but we never know whether it is distended to exactly the same degree as in the living organism. Our measurement of the proximal aorta is perhaps a little better because there we were able to get the diameter from the size of the probe which accurately fit the living, pulsating aorta.

SURGICAL STUDIES IN HEMATOPOIESIS

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(From the Department of Surgery, University of Oregon Medical School)

MANY attempts have been made to design a technique for the cultivation of bone marrow tissue. To date there is no method which would correspond to a true tissue culture of bone marrow in the sense that it would keep bone marrow alive indefinitely in the form in which it was explanted. Some bone marrow cells can be kept viable for long periods by a number of methods but such "cultures" are usually not representative of the tissue which was explanted. The main difficulty lies in the fact that bone marrow represents a mixed population of cells which differ in their nutritional requirements and in which both cell multiplication and differentiation occur. The correct balance of these requirements has yet to be reproduced in long-term in vitro cultures.[2]

For some time now we have been interested in the possibility of designing a reproducible in vivo culture of marrow. Among the requirements which must be fulfilled before success is claimed for an in vivo culture method are the long-term growth of myeloid, erythroid, and megakaryocytic elements, and evidence that the proliferating marrow cells are the progeny of the marrow inserted and not of the host.

A technique developed in this laboratory permits the long-term growth of autologous bone marrow implants in the dog.[1] Although we have not identified the "stem" cell, data presented in this report support the concept that the proliferating marrow cells in this preparation are the descendants of the marrow inserted.

METHOD

With Nembutal anesthesia, operations were carried out on young mongrel dogs weighing 35 to 45 pounds. An incision was made over the left eight or ninth rib area in the direction of, and down to, the rib. After reflection of the periosteum, 10 cm. segments of 2 ribs just posterior to the costochondral junction were excised and covered with sponges soaked in normal saline solution. The ribs were then refrigerated at 4° C. while the chest wound was sutured. At this point the animal was given 150 r total body irradiation in air using a Thoraeus 2 filter, 0.4 tin, 0.25 copper, 1 aluminum: distance of 76 cm. 15.5 r for 1 minute.

Four groups of dogs with autologous transplants were evaluated.

Group I.—Following irradiation the abdomen was opened through a midline epigastric incision. The rib portions were divided into halves 5 cm. long and further bisected longitudinally with bone shears. Twenty dogs were studied in this group. In 12 of these, 4 segments of rib were inserted into the splenic substance from the upper edge at right

Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

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Fig. 1.

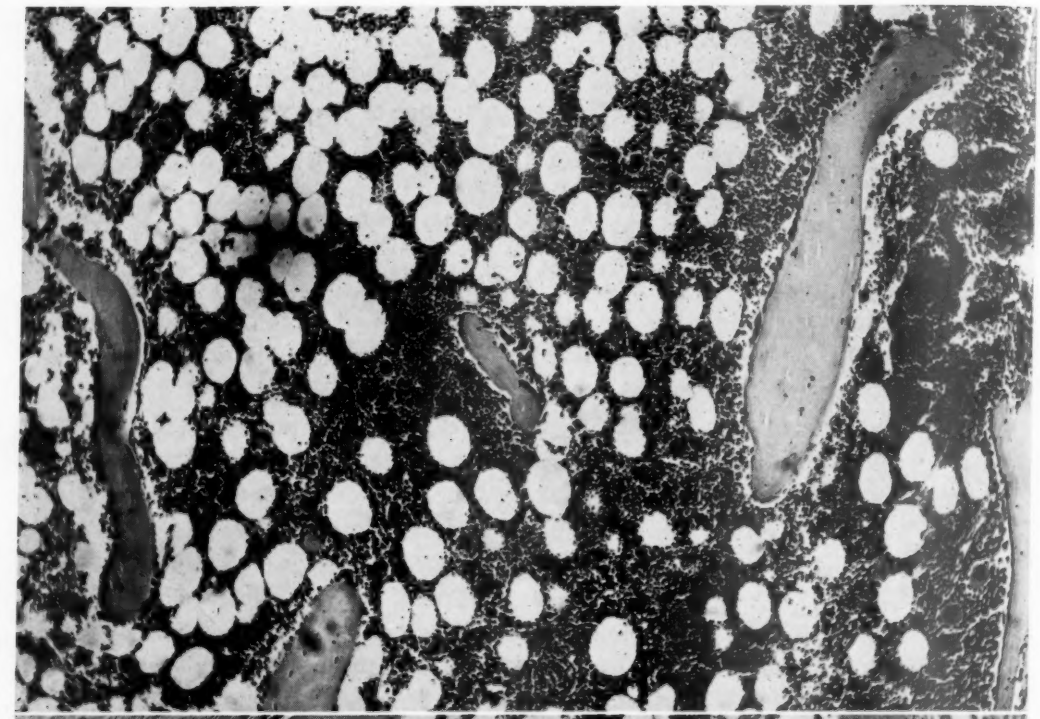

Fig. 2.

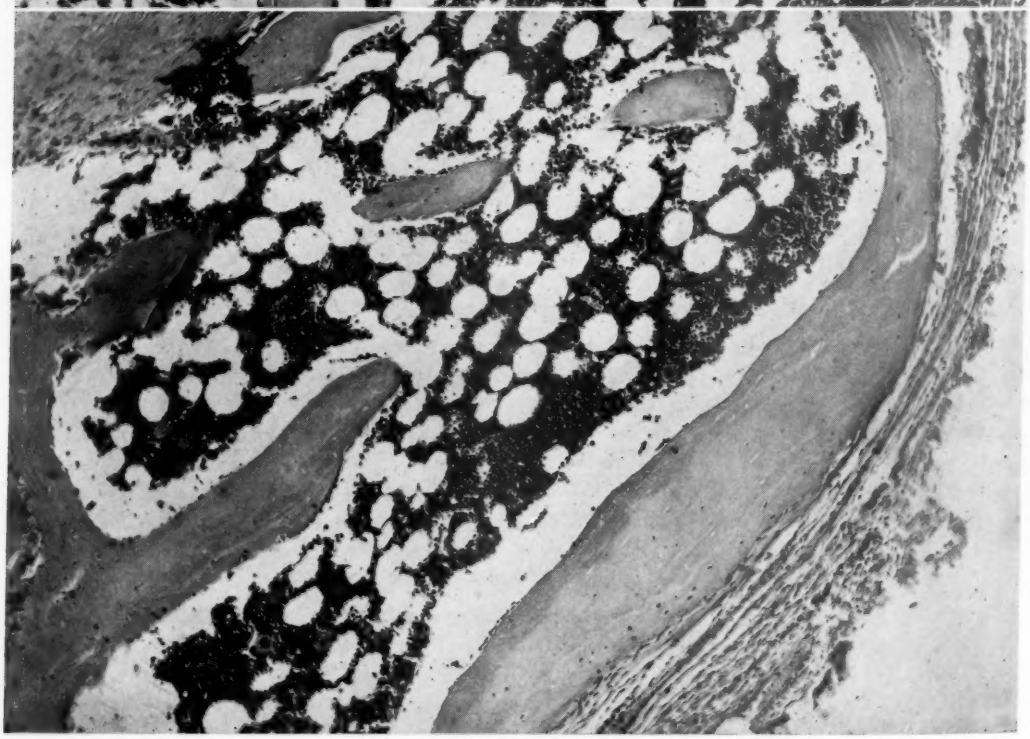

Fig. 1.—Rib with marrow removed from spleen 2 years after insertion. Osteoblasts are continuing to lay down new bone. (Giemsa; original magnification ×100.)

Fig. 2.—Rib with marrow removed from omentum 6 months after insertion. (Giemsa; original magnification ×100.)

angles to the long axis of the spleen and 4 segments were wrapped in omentum and held encased by a single chromic suture. Serial investigations were carried out by removing the implanted ribs from the spleen or omentum after planned intervals and studying the cytology and histochemistry of the contained marrow.

Group II.—In 8 dogs split rib segments were inserted into the spleen, omentum, liver, kidney, subcutaneous tissue of the anterior abdominal wall, and the rectus abdominis muscle. These animals were sacrificed 6 months later at which time the spilt ribs were removed and fixed in Zenker's solution for histologic evaluation.

Group III.—Two split rib segments with contained marrow were inserted into one end of the spleen in 12 dogs. In 6 of these dogs, the abdomen was reopened on the fourth postoperative day and in 6 it was reopened on the eighth postoperative day. At operation 1 split segment of rib was removed and reinserted into the opposite pole of the spleen. The remaining rib in the spleen, carefully marked with a silk suture, was left as a control. The splenic specimens were removed for histologic study 7 weeks after operation.

Group IV.—The influence of refrigeration on the growth of autologous marrow in the spleen was investigated in 2 dogs. One split segment of rib was threaded into the spleen immediately after x-ray and the remaining rib portions were refrigerated at 4° C. Specimens refrigerated for 2 and 14 days, respectively, were inserted into the splenic substance. All the implants were removed 8 weeks after the initial procedure.

RESULTS

Group I.—The results of this group of animals and photomicrographs of the sequence have been previously reported.[1] A brief summary follows.

Serial investigations of the transplants in the spleen and omentum showed that 6 hours after implantation the marrow cells were disintegrating. Within 72 hours the specimens were markedly hypocellular, exhibiting for the most part only scattered, primitive-looking mononuclear cells and small groups of osteoblasts. The osteoblasts were identified by their intense alkaline phosphatase activity. Specimens obtained on the third to tenth postoperative days presented conditions similar to that of the 72-hour marrow. On the eleventh to fifteenth days immature myeloid, erythroid, and platelet elements could be found. Four to 6 weeks after implantation full-blown marrow proliferation was in evidence. In 1 animal, 2 years after the original procedure (the longest these animals have been followed to date), the split rib segment was intact and the marrow growth good (Fig. 1).

Group II (Table I).—Figs. 2-5 are characteristic of the bone marrow growth in omentum, kidney, liver, and muscle at the end of 6 months. Subcutaneous tissue did not support growth.

TABLE I. ECTOPIC BONE MARROW GROWTH IN DOGS

SITE OF IMPLANTATION	NUMBER OF ANIMALS	PERIOD OF OBSERVATION (MO.)	MARROW GROWTH		
			EXCELLENT	FAIR	NO GROWTH
Spleen	20	6-24	20		
Omentum	20	6	20		
Kidney	8	6		4	4
Liver	8	6		3	5
Muscle	8	6		2	6
Subcutaneous tissue	8	6			8

Fig. 3.

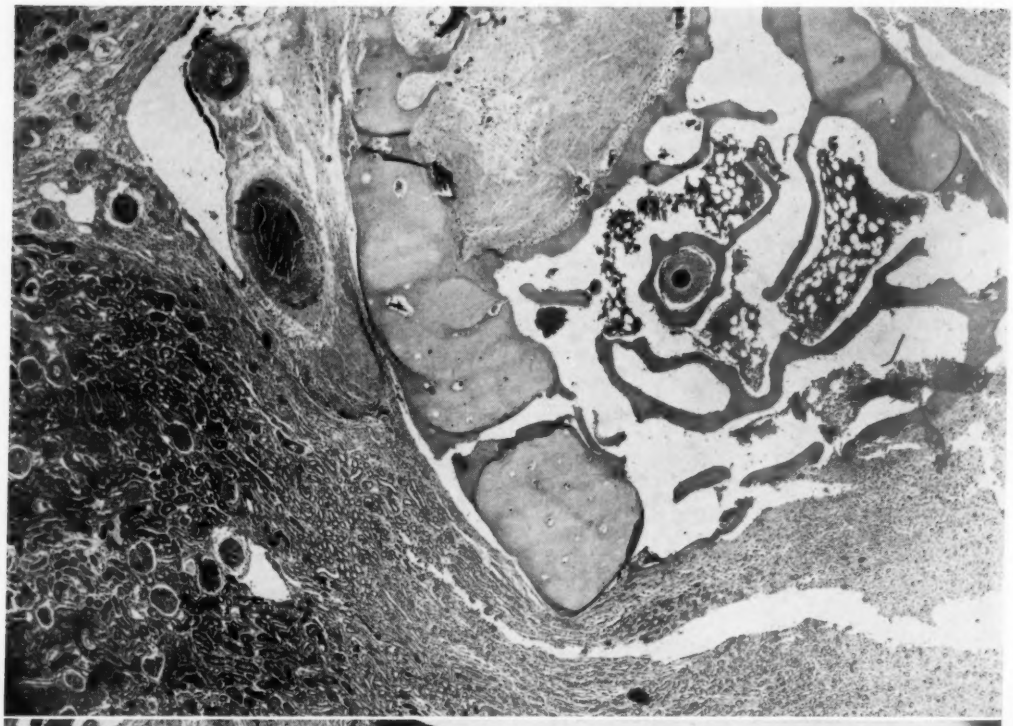

Fig. 4.

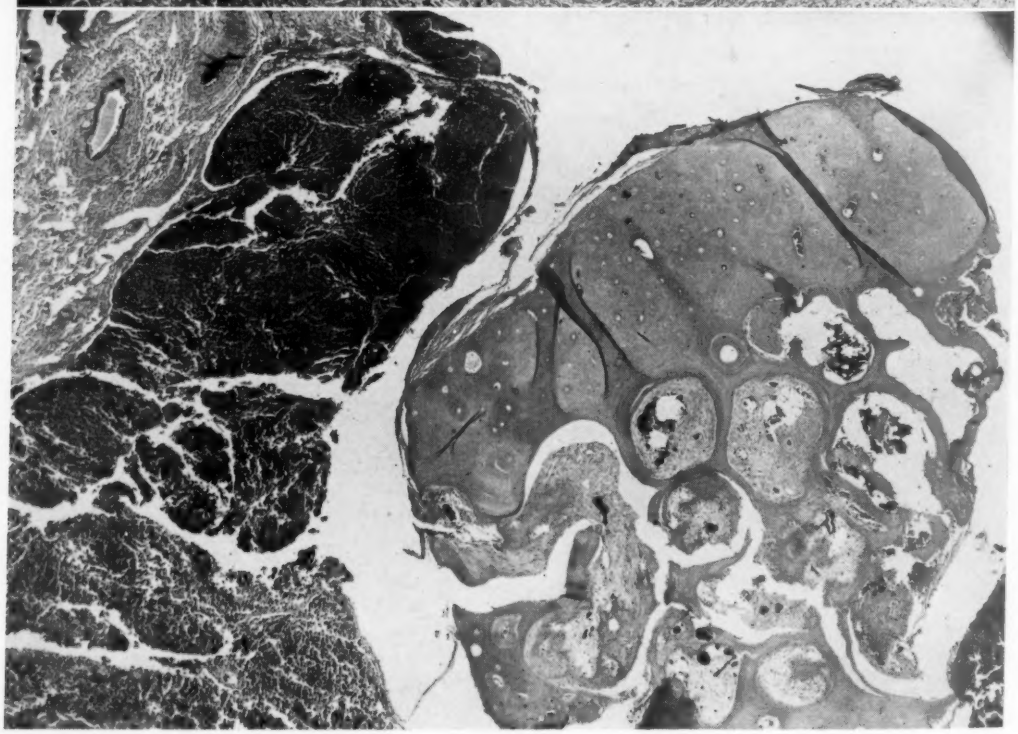

Fig. 3.—Specimen of rib, marrow, and surrounding kidney tissue excised 6 months after insertion. (Giemsa; original magnification ×30.)

Fig. 4.—Section of bone, contained marrow, and encasing liver 6 months after rib threaded into this area. Proliferation of marrow is scanty. (Giemsa; original magnification ×30.)

Fig. 5.

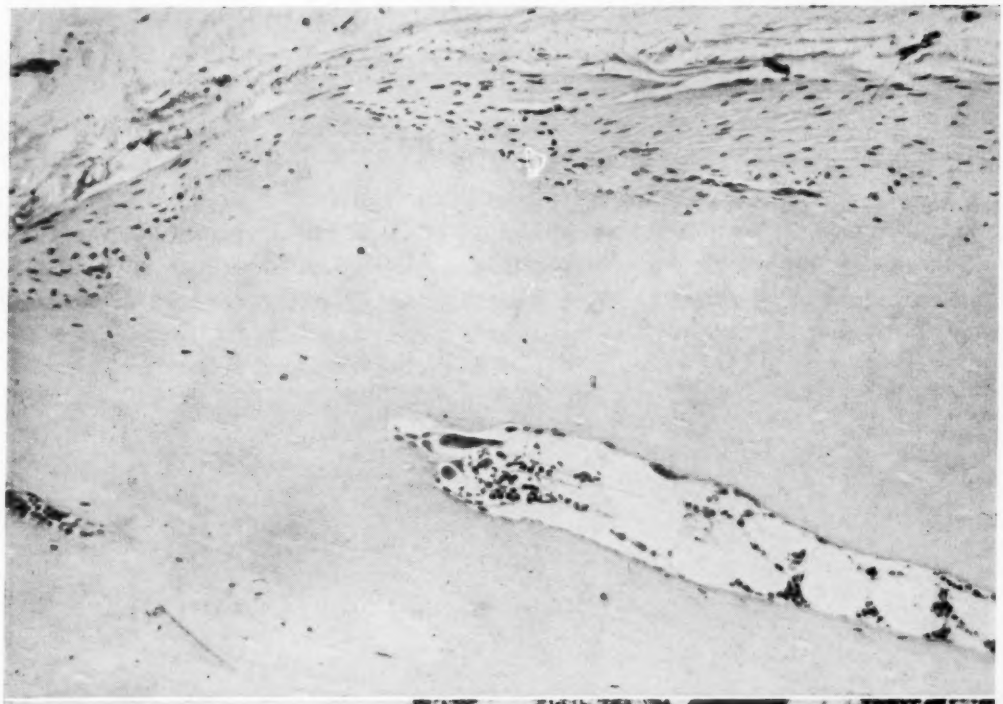

Fig. 6.

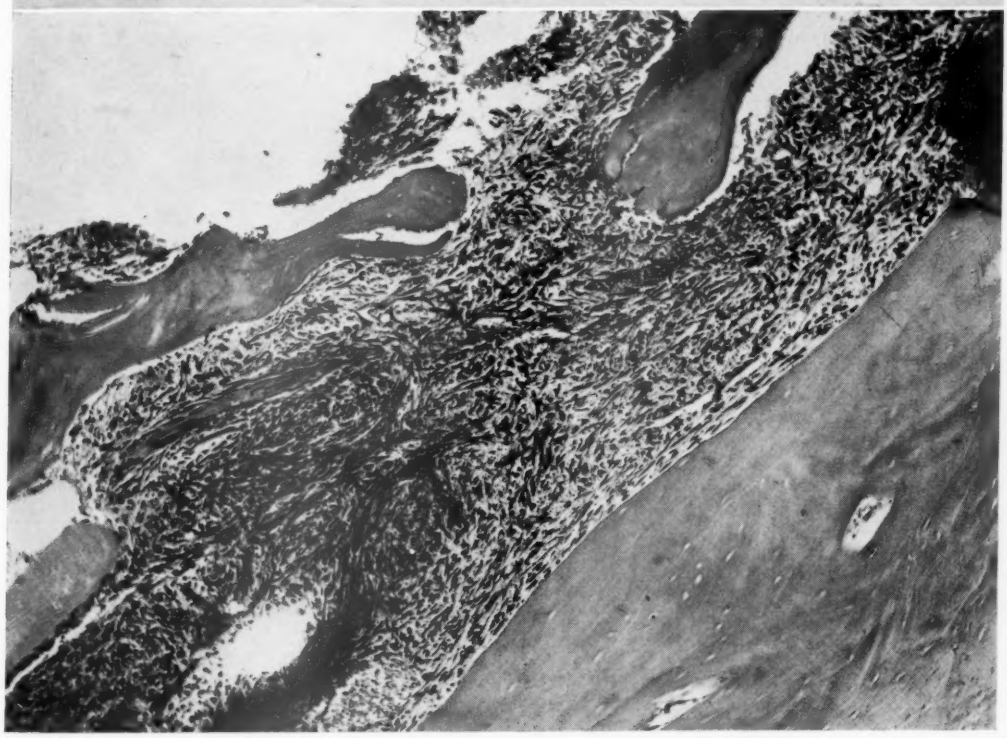

Fig. 5.—Intramusclar rib with limited marrow proliferation 6 months after insertion into the rectus muscle. (Giemsa; original magnification ×115.)

Fig. 6.—Fixed section of rib with fibrous tissue removed 8 days after insertion into spleen. This was followed by marrow growth in 7 weeks. (Giemsa; original magnification ×100.)

In general the gross appearance and size of the split rib in the spleen remained unchanged from the control. The ribs extracted from the liver, omentum, and muscle were somewhat atrophic and the bony portion of the subcutaneous specimen had been practically replaced by fibrous tissue. The split rib inserted into the substance of the kidney was larger than the control and showed multiple bony projections not previously present.

Group III.—In this group the ribs were transferred to other sites in the spleen during the hypocellular phase of splenic proliferation at either the fourth or the eighth day after insertion. All contained numerous primitive-appearing mononuclear cells. They were removed from their splenic bed 7 weeks later.

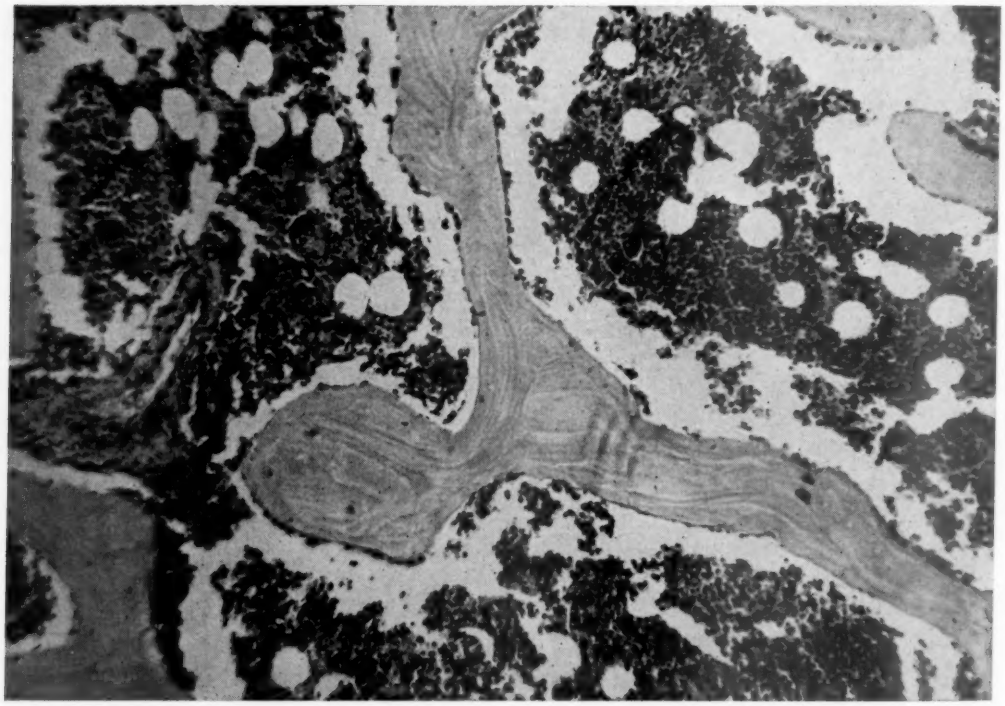

Fig. 7.—Rib with good proliferation of marrow 7 weeks after specimen shown in Fig. 6 inserted. (Giemsa; original magnification ×115.)

Among the group transferred at 4 days, 1 demonstrated good marrow growth, 4 scanty proliferation, and 1 no growth. In the group transferred 8 days after splenic insertion and evaluated 7 weeks later, 4 were characterized by marked marrow growth, and 2 showed scanty proliferation.

Fig. 6 shows a fixed section of an 8-day specimen. Fibrous tissue is prominent. This same rib 7 weeks later, Fig. 7, showed excellent proliferation of marrow.

Group IV.—The growth potential of marrow refrigerated for 48 hours was unchanged from the control. Two weeks of refrigeration was not followed by marrow proliferation, although the refrigerated rib itself remained viable as evidenced by new bone formation.

DISCUSSION

In this preparation, the spleen of the dog proved to be the most satisfactory locus for ectopic cultures of bone marrow. The omental site is considered not quite as favorable as the spleen because, although growth was excellent, atrophy of bone developed at 6 months. Viable bone is probably necessary for the growth of marrow in this preparation since boiled bone inserted into the spleen did not grow marrow. The kidney, liver, and muscle proved to be less reliable sites of marrow growth than either the omentum or spleen, and the subcutaneous tissue did not support prolonged marrow proliferation. The authors are able to offer only speculation as to the specific aspects of the environment which influence bone marrow growth in the various ectopic locations which were evaluated. The real environmental aspects remain obscure.

Data derived from the transfer experiments deserve some explanation. In this group, ribs which were markedly hypocellular except for the presence of mononuclear cells, osteoblasts, and lymphocytes were inserted into other locations. When the transfer was carried out 4 days after implantation into the spleen, the subsequent marrow growth was scanty. When the transfer was completed 8 days after implantation, good marrow proliferation developed. Mature marrow, inserted into the splenic site, disintegrates. If the blood supply to the implant is not further interrupted by transfer of the rib to another site, regeneration occurs. Transfer of the implant late in the hypocellular period is apparently followed by better growth than transfer completed early in this phase.

A possible explanation might be that a series of inhibitors to cell growth, remaining after the degeneration of mature cells, interferes with "stem" cell proliferation. This concentration may be higher at 4 days after implantation than at 8 days since most of the disintegrated mature cells seem to have undergone phagocytosis by this time.

In the transfer experiments the sequence between implantation and growth was similar whether the original specimen showed good marrow or whether it was in the late hypocellular phase. A series of experiments is now underway with tritiated thymidine to shed further light on the question of the origin and nature of the cell which forms marrow in this preparation. When the tritiated thymidine studies have been completed, we should have conclusive data. The evidence from another line of investigation indicates that the implant is the source of the "stem" cell since ribs refrigerated for over 2 weeks remain intact and continue to lay down new bone but do not form marrow.

A reproducible technique for the ectopic culture of bone marrow in the dog has been developed. The marrow which proliferates in this preparation appears to come from cells derived from the implant itself.

CONCLUSIONS

1. Autologous bone marrow contained in split rib segments will proliferate well in the dog spleen for periods of at least 2 years. The omentum is a favorable site. Growth in the kidney, liver, and muscle was not consistent. Substantial growth has not been observed in subcutaneous tissue.

2. Ribs implanted during the hypocellular phase will grow marrow in the same time sequence as ribs implanted with mature marrow.

3. Evidence from these experiments is consistent with the fact that the marrow which grows in the implanted rib descends from the implant itself.

REFERENCES

1. Jacob, S. W., Moloney, W. C., Barsamian, E. M., Owen, O. E., and Dunphy, J. E.: Histologic Sequences Following Implantation of the Autologous Canine Rib Into the Spleen, Surg. Gynec. & Obst. 109: 697, 1959.
2. Lajtha, T. G.: The Culture of Bone Marrow Cells In Vitro, Brit. M. Bull. 15: 47, 1959.

COSTAL INTRAOSSEOUS VENOGRAPHY: AN AID IN MANAGEMENT OF INTRATHORACIC NEOPLASIA

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MANY diagnostic procedures are utilized by physicians to determine the type, size, and extent of intrathoracic neoplasms. None of these is uniformly helpful in determining the resectability of such lesions. Lessman and Schobinger[3] have demonstrated the ease with which radiographic visualization of the azygos and hemiazygos systems may be obtained by injection of contrast material into the marrow cavity of a rib. The contrast medium diffuses rapidly from the marrow cavity into the systemic venous circulation and delineates abnormal vascular channels in and about certain pathologic processes. We have utilized this method to determine resectability of pulmonary and esophageal neoplasms. This report concerns our experience with costal intraosseous venography in 40 patients.

The azygos and hemiazygos veins lie in close proximity to the hilum of the lung. Obstruction of these venous systems by infiltrating neoplasms produces a reversal of blood flow within them. This abnormality is easily detected by costal intraosseous venography. The change in blood flow is usually manifested radiographically by visualization of multiple collateral veins including the paravertebral, the intervertebral, and the veins of the spinal cord. Venous pathways as far distant as the lumbar veins have been identified in some patients. When neoplastic infiltration produces obstruction of the azygos or hemiazygos veins or of the superior vena cava of a degree which is detectable by this technique, it is felt that resectability for cure is precluded for that patient.

ANATOMY

Contrary to the findings of Schobinger[7] we have not found the normal pattern of the azygos and hemiazygos venous systems to be constant. We have observed a number of variations which are not clearly described in anatomy textbooks or in the clinical literature, and which may be divided into 5 categories. These are outlined in Figs. 1-5. The first is the most common and the fifth, namely, that of azygos lobe syndrome, is the least common.

The crossover or communicating vein between the hemiazygos and azygos systems is inconstant in level. This area of communication can be found anywhere from the level of D_9 to as high as D_3. Multiple connections between the azygos and hemiazygos veins are common. The so-called azygos bulb, or the

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Fig. 1.

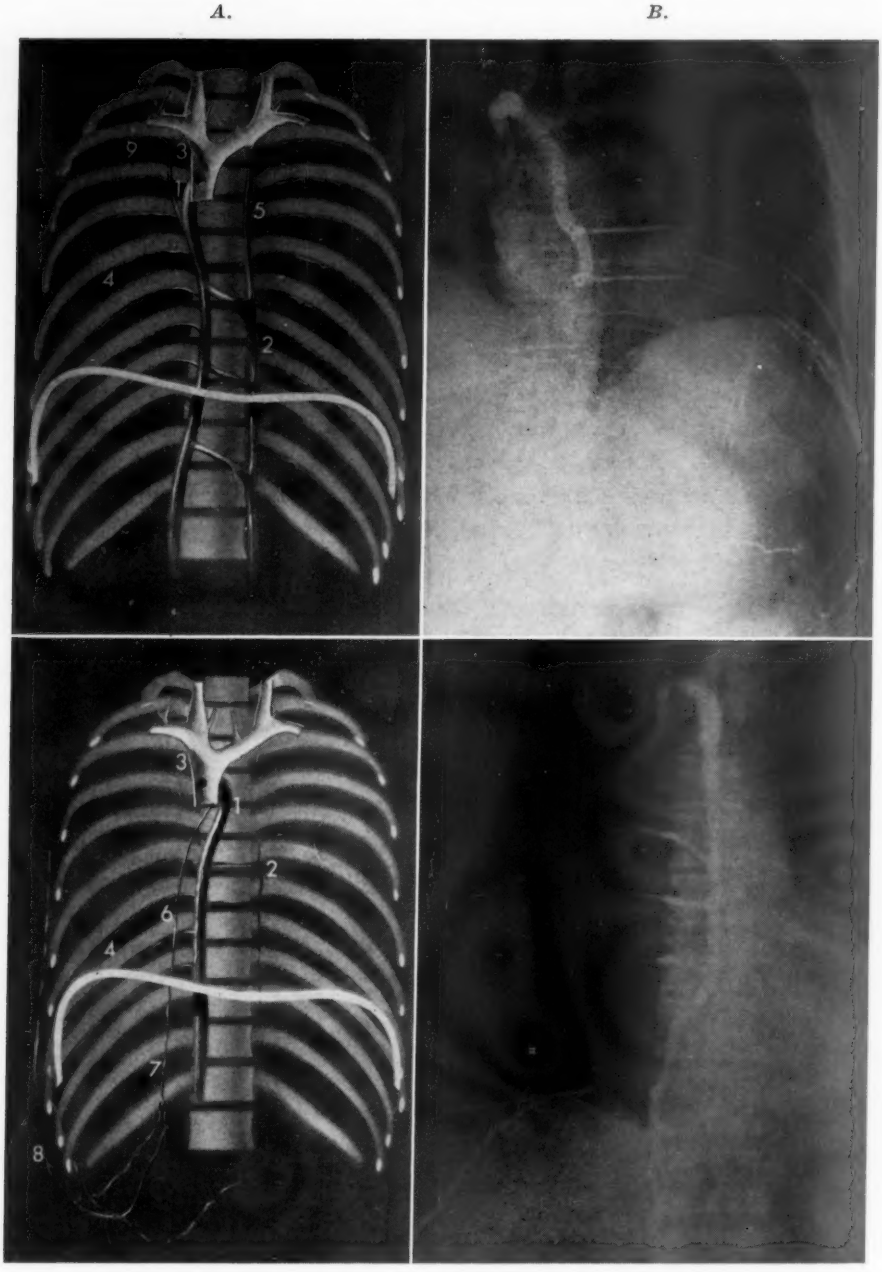


Fig. 2.

Fig. 1.—*A*, Usual anatomic configuration of the azygos and hemiazygos systems. This diagram shows the most common level of the venous communications between the 2 systems. (1) Azygos vein, (2) hemiazygos vein, (3) superior vena cava, (4) intercostal vein, (5) azygos tertius vein, (9) superior intercostal vein. *B*, Normal venogram made after left-sided injection. This shows the intercostal veins feeding into the hemiazygos vein. This in turn communicates with the azygos system. This is the same anatomic configuration which is portrayed in Fig. 1, *A*.

Fig. 2.—*A*, This is a common variant of the azygos system. (1) Azygos vein, (2) hemiazygos vein, (3) superior vena cava, (4) intercostal vein, (6) cardiophrenic vein, (7) musculophrenic vein, (8) lateral thoracic vein. *B*, Normal venogram with anatomic configuration similar to that illustrated in Fig. 1, *A*.

Fig. 3.

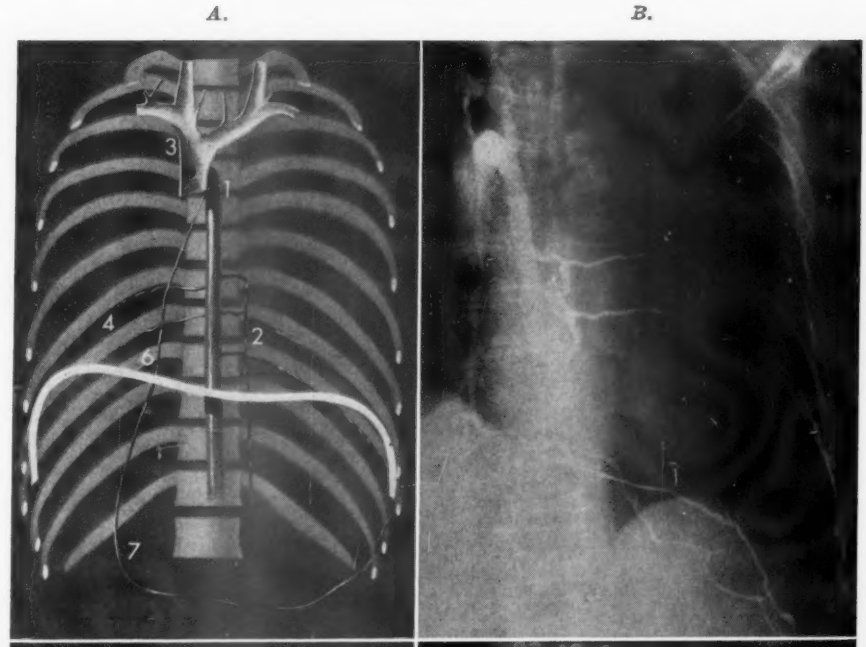


Fig. 4.

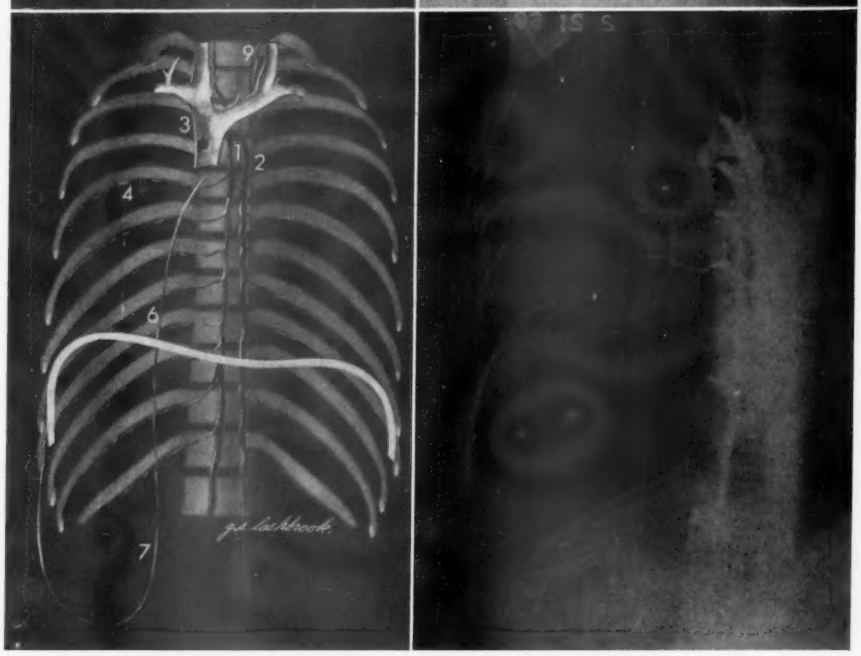
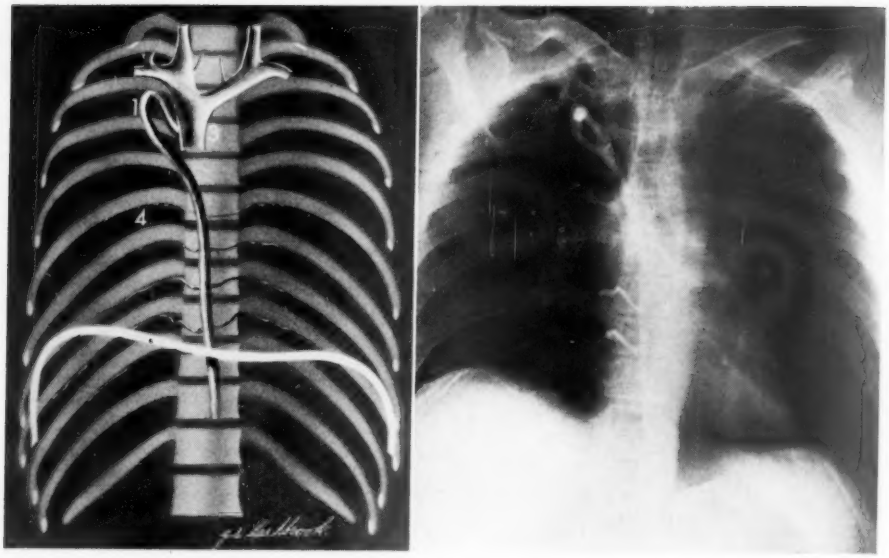


Fig. 3.—*A*, Another common variant of the azygos system. (1) Azygos vein, (2) hemiazygos vein, (3) superior vena cava, (4) intercostal vein, (6) cardiophrenic vein, (7) musculophrenic vein. *B*, Normal venogram with the anatomic configuration which is illustrated in *A*. Note particularly the cardiophrenic vein.

Fig. 4.—*A*, Double azygos system. (1) Azygos vein, (2) hemiazygos vein, (3) superior vena cava, (4) intercostal vein, (6) cardiophrenic vein, (7) musculophrenic vein, (9) azygos tertius vein. *B*, Venogram which shows a double azygos system. Note that the azygos and hemiazygos veins are of equal size with multiple communicating veins. Note also the cardiophrenic vein. Patient has advanced pulmonary tuberculosis without obstruction of the azygos system.



A. *B.*

Fig. 5.—*A*, Azygos-lobe type configuration. (1) Azygos vein, (3) superior vena cava, (4) intercostal vein. *B*, Venogram which shows patent azygos vein, with typical azygos lobe configuration.

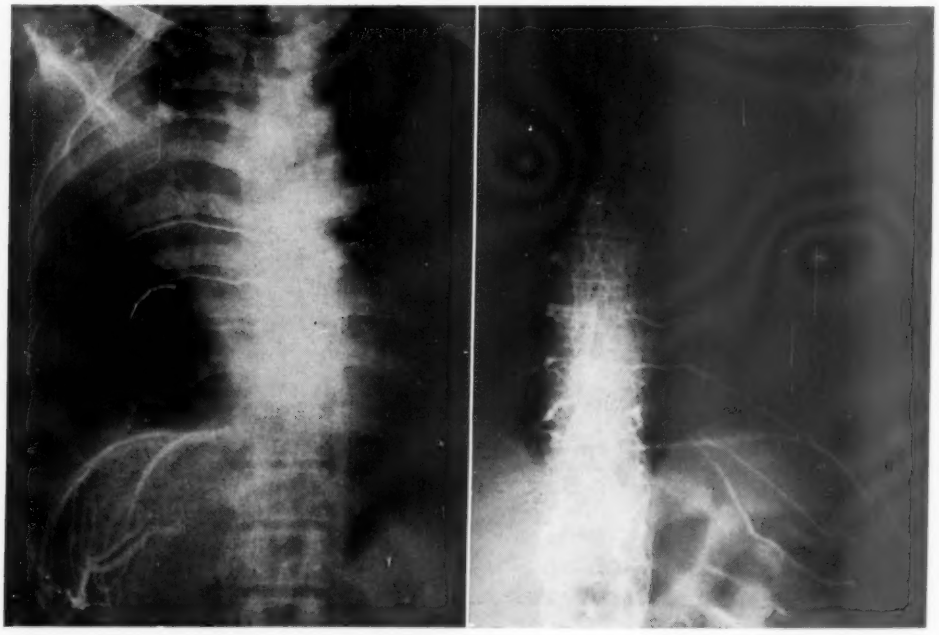


Fig. 6. Fig. 7.

Fig. 6.—Abnormal venogram which shows complete block of the azygos system. Note collateral vessels over the lateral thoracic wall and reversal of blood flow down the paravertebral veins in the lumbar region. Patient had an unresectable squamous cell carcinoma originating in the right upper lobe.

Fig. 7.—Abnormal venogram which shows complete block of the azygos system secondary to an unresectable squamous cell carcinoma of the left lower lobe. Note reversal of blood flow with fill of intervertebral and paravertebral veins. This type of pattern is typical of complete block of the azygos system.

junction of the azygos vein with the superior vena cava, is also found at an inconstant level and may be anywhere from the level of D_2 to D_5. The finding of the cardiophrenic vein, even on normal venograms, has made it necessary to include this in our anatomic diagrams to avoid confusion. This vein communicates with the intracostal veins and is readily filled by intramedullary injection in the region of the ninth or tenth ribs. The internal mammary vein has been visualized on several occasions in venograms which are otherwise normal. This finding should not be misinterpreted as representing an abnormality. The superior intercostal vein and the azygos tertius vein will not be visualized unless there is complete obstruction of the azygos or hemiazygos systems. These are included in the diagrams for the sake of completeness.

TECHNIQUE

We have utilized a technique similar to that described by Lessman, Schobinger, and Lasser.[4] This procedure is first explained to the patient who is forewarned to expect mild pain and a sensation of pressure at the time of injection. Appropriate studies are performed prior to the study to exclude a bleeding tendency, for there is a definite though minimal danger of hemorrhage following this procedure. The patient is premedicated with Demerol and a barbiturate 1 hour prior to examination. In the x-ray department he is placed in a supine position on the x-ray table, and a test film is taken using the same technique one would employ for radiologic examination of the dorsal spine. This permits sufficient contrast between the contrast material and the prominent bony structures of the thorax. Lesser penetration of x-ray than this will give insufficient contrast between the structures, and thus failure of visualization of the venous systems. Following proper positioning and adjustment of technical quality of the film for proper exposure, the skin over the tenth rib in the midaxillary line is infiltrated with 1 per cent Xylocaine. The infiltration is carried down to and including the periosteum of the rib. We have preferred to use a short No. 18 caliber spinal needle in making the injection as it is easily introduced into the marrow cavity, with little tendency to penetrate completely through the rib. The needle tip is placed in the marrow cavity by slanting it obliquely over the course of the rib and using a slight rotary motion as gentle pressure is applied to the needle. A successful marrow tap is heralded by the ability to aspirate bone marrow and blood from the rib. If the needle is inadvertently pushed entirely through the rib, an adjacent rib should be chosen for the injection. If this precaution is not taken, the contrast medium will escape from the previously made needle tract. After a satisfactory marrow tap has been achieved, 2 to 5 ml. of 1 per cent Xylocaine are injected directly into the marrow cavity. This provides sufficient anesthesia to permit completion of the procedure with little or no pain. The patient is then instructed to hold his breath in a mid-respiratory phase. In this manner, there is no tendency to reproduce a Valsalva maneuver which might potentially obstruct the venous flow. Following this, 20 c.c. of 50 per cent Hypaque is rapidly injected into the marrow cavity and a single film is taken immediately upon the conclusion of the injection.

The side effects of this procedure have been minimal. On several occasions, dye has diffused out of the needle tract into the pericostal area. This has not been a serious problem and has caused the patient very little discomfort. There has been no incident of pneumothorax or of significant hemorrhage.

ANALYSIS OF MATERIAL

In this series 44 examinations were made in 40 patients. Contralateral examinations were performed in 4 patients to clarify abnormalities found on the initial venogram.

The lesions for which the examinations were indicated may be divided into 4 categories: (1) intrathoracic inflammatory lesions, (2) neoplasms of the lung, (3) neoplasms of the esophagus, and (4) miscellaneous intrathoracic abnormalities.

Intrathoracic Inflammatory Lesions (7 Patients, Table I).—Six venograms were made in patients with active far-advanced pulmonary tuberculosis. In no instance, despite extensive disease, was there a demonstrable block of the azygos or hemiazygos systems. These examinations have served to supplement our knowledge of the normal radiologic anatomy. One patient with extensive actinomycosis was examined and found to have a deformity of the azygos vein secondary to advanced lymphadenopathy. This distorted the azygos vein but did not occlude it.

TABLE I. INFLAMMATORY

CLINICAL ENTITY	NO. OF PATIENTS	VENOGRAPHIC FINDINGS		
		NORMAL	DISTORTION OF VEINS	BLOCK
Tuberculosis	5	5	0	0
Tuberculosis and portal hypertension	1	1	0	0
Actinomycosis	1	0	1	0
Totals	7	6	1	0

Neoplasms of the Lung (21 Patients, Table II).—Thirteen of these patients were found by venography to have complete block of the azygos or hemiazygos system. In 12 of these the findings were confirmed at either operation or autopsy; in the other, the presence of a complete block of the azygos vein denoting a nonresectable neoplasm was confirmed presumptively by a venous angiogram (Fig. 8). Five patients in this category demonstrated normal venograms and were found at the time of operation to have lesions which were resectable with hope for cure. Two patients in this category had normal venograms which were misleading. At the time of operation 1 of these patients had extension to the anterior chest wall, and the other patient had tumor erosion of the pulmonary artery. It was not felt technically feasible to resect the lesions in either of these 2 patients. These 2 examinations represent our only false-negative findings. The 1 additional patient in this category was shown by venography to have distortion of the arch of the azygos vein. At the time of operation an inflammatory node was found in this area. The vein was not obstructed and the patient's tumor was resected with hope for cure.

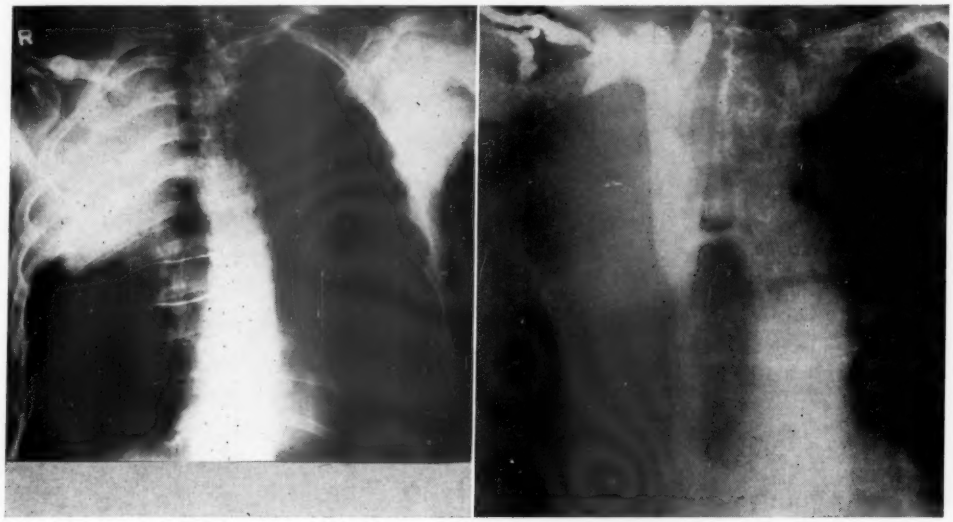
TABLE II. NEOPLASMS OF THE LUNG

CLINICAL ENTITY	NO. OF CASES	VENOGRAPHIC FINDINGS		
		NORMAL	DISTORTION	BLOCK
Carcinoma of lung	21	7*	1†	13

*Two of these were false normals.
†Distorted by inflammatory node but not blocked.

Cancer of the Esophagus (4 Patients, Table III).—Four patients with proved carcinoma of the esophagus were examined and the venograms were found to be normal in all of them. Two of the patients were inoperable because of extension of tumor about the aortic arch. Another had a lesion which was nonresectable because of invasion of the anterior mediastinum.

The last patient in this category with proved carcinoma of the esophagus was examined at the termination of cobalt irradiation therapy and again the venogram was normal. While the number of patients in this category is small, our data suggest that venography is of no value in establishing resectability in patients with esophageal carcinoma. We believe, however, that more data will be necessary before a definite conclusion can be drawn.



A. *B.*

Fig. 8.—*A*, Abnormal intracostal venogram which shows lack of visualization of the azygos vein due to reversal of blood flow secondary to an unresectable bronchogenic carcinoma of the right upper lobe. *B*, Venous angiogram in same patient. This suggests direct invasion of the superior vena cava and azygos vein by neoplasm. The reversal of blood flow in the azygos system shown in *A* is substantiated. This patient has been erroneously judged to be a candidate for operation on the basis of the standard diagnostic criteria.

Miscellaneous (8 Patients, Table III).—We include in this category all other patients examined by this technique, some of whom present significant findings. It was necessary to ligate the azygos vein during the course of segmental resection for pulmonary tuberculosis

TABLE III. MISCELLANEOUS

CLINICAL ENTITY	NO. OF PATIENTS	VENOGRAPHIC FINDINGS		
		NORMAL	DISTORTION	BLOCK
Portal hypertension	1	1	0	0
Superior vena caval obstruction	1	0	0	1*
Chronic pneumonitis	1	1	0	0
Bullous emphysema	1	1	0	0
Gastric carcinoma with pulmonary metastasis	1	1	0	0
Azygos lobe syndrome	1	1	0	0
Azygos vein ligation secondary to segmentectomy	2	0	0	2
Totals	8	5	0	3

*Secondary to tuberculous pericarditis.

in 2 patients. In both of these, venography revealed a reversal of blood flow comparable to that seen in patients with infiltrating tumors. One patient in this category had a consolidating lesion of the lung which was thought clinically to be a carcinoma. However, this could not be proved by bronchoscopy, cytologic washings, or scalene node biopsy. The venogram was entirely normal and operation was not advised. Long-term antibiotic therapy resulted in complete regression of the lesion, which led to the final diagnosis of chronic pneumonitis. Two patients in this category are of interest. One showed a complete block of the azygos system with reversal of flow which substantiated the clinical diagnosis of severe superior vena cava thrombosis. This was felt clinically to be due to tuberculous pericarditis. One

patient in this series had massive bullous emphysema. Venography in this patient, as was expected, was completely normal. Another patient in this series had pulmonary metastases from a primary carcinoma of the stomach. These were diffuse and in the parenchyma of the lung. Venography in this patient was completely normal as the infiltration was distal rather than in the central and mediastinal areas of the thorax.

We have carried out azygos venography in 2 patients with proved portal hypertension. One of these patients is included in the first category since he also had far-advanced pulmonary tuberculosis. Both of these patients had normal venograms and in neither patient were we able to reproduce the findings of multiple collaterals and failure of visualization of the entire azygos system as has been shown by Schobinger.[7]

SUMMARY AND CONCLUSIONS

Costal intraosseous venography has been performed 44 times in 40 patients. We have experienced no complications following these procedures. Variations in the normal anatomy of the azygos and hemiazygos system have been clarified and described. We feel that this procedure is primarily useful as an aid in predicting resectability in patients with pulmonary neoplasms. It is significant that extensive inflammatory diseases do not produce changes similar to those produced by neoplasms. In our opinion, costal intraosseous venography is a safe procedure which should be included in the armamentarium of all those dealing with pulmonary neoplasia.

REFERENCES

1. Bachman, A. L.: Radiographic Diagnosis of Mediastinal Involvement in Carcinoma of the Lung, J. Mt. Sinai Hosp. **24**: 4-13, 1957.
2. Burke, D. T., and Goldberg, L.: Azygos Vein in Tomography, Canad. M. A. J. **60**: 271-272, 1949.
3. Lessman, F. P., and Schobinger, R.: Intra-osseous Venography in Portal Hypertension, Acta radiol. **51**: 95, 1959.
4. Lessman, F. P., Schobinger, R., and Lasser, E. G.: Intra-osseous Venography, New York J. Med. **55**: 2938, 1955.
5. Lessman, F. P., Schobinger, R., and Lasser, E. G.: Intra-osseous Venography in Skeletal and Soft Tissue Abnormalities, Acta radiol. **44**: 397-409, 1955.
6. Salzmann, F. A., and Wise, R. E.: Intra-osseous Venography, S. Clin. North America **36**: 801, 1956.
7. Schobinger, R.: Costal Intra-osseous Venography in the Diagnosis of Portal Hypertension, Gastroenterologia **88**: 21-31, 1957.
8. Schobinger, R.: Intra-osseous Venography, Cleveland Clin. Quart. **23**: 155-157, 1956.
9. Schobinger, R., and Lessman, F. P.: Intra-osseous Venography—Its Value to the Surgeon, S. Forum **7**: 459-463, 1957.
10. Schwartz, S., Handel, J., and Candel, S.: Azygography, Radiology **72**: 338, 1959.
11. Tori, G.: Radiological Demonstration of the Azygos and Other Thoraco-abdominal Veins in the Living, Brit. J. Radiol. **27**: 16-22, 1954.

DISCUSSION

DR. HERBERT SLOAN (Ann Arbor, Mich.).—When this work first started, I viewed it with sort of a tolerant eye. It seemed something to keep the young men out of mischief. As time has gone on, I have become greatly impressed with the value of this examination in weeding out patients who should not undergo thoracic exploration. There is little to be gained from exploratory procedures for patients with irremovable carcinoma of the lung. We had originally thought that venous angiocardiography would give us valuable information about resectability of carcinoma of the lung but, disappointingly, it has not. This added diagnostic tool, which is simpler than angiocardiography and which has been safe in the hands of Dr. Fry and his co-workers, has shown a startling correlation with the things we have found at operation. We believe it will allow us to avoid many unnecessary thoracotomies. We plan to use the technique more frequently.

EXPERIENCES WITH A SIMPLIFIED METHOD OF SMALL ORGAN PERFUSION

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THE purpose of this communication is to describe a method of studying a surviving tissue which is maintained in vitro by perfusion. The problems discussed here apply to the perfusion of any piece of tissue which has a small artery available for cannulation.

Our ultimate aim in carrying out perfusion experiments, on the ovary in particular, has been to determine the chemical nature of the estrogens secreted by the isolated human ovary under various conditions. We shall not deal with the chemical problems, which have been our main difficulty as well as the major concern of our efforts, except to point out that one can ordinarily expect to recover not much more than 100 mcg.—one-tenth of a milligram—of estrogen in a 24-hour urine specimen obtained from a nonpregnant woman with active ovaries. In the absence of radioactive tracer substrates there is thus little likelihood that chemically identifiable estrogen will be manufactured by a slice or homogenate preparation, and only a hope that a perfusion may do so.

PERFUSION MEDIUM

The choice of a medium for perfusion is of the first importance. Why not use whole blood, as in the many perfusion techniques being used today in surgery? The reasons are related both to technicalities of perfusion and to those of the chemical determinations in question. The latter difficulty was encountered in our initial attempt to perfuse Mrs. Z's ovary with blood obtained from Mrs. Z at operation, when it became quite obvious that a blank determination of hormone on Mrs. Z's blood, simultaneously obtained and not used in perfusion, would have to be made. This means not a simple colorimetric determination but a long series of chemical steps. The methods involved are still in process of elaboration and are worth undertaking only where the conduct of the entire experiment ensures freedom from artefact and ambiguity. It is also clear that the use of blood from another source, human or animal, presents even more difficulties. In addition to this, there are the technical problems of perfusion with blood. The flow through an ovary can be expected to be not more than 0.1 ml. per gram of wet weight per minute. A total flow of only 1 or 2 ml. per minute means that the red cells tend to settle out by gravity in the tubing. Red cells of a compatible type by cross-match must, of course, be used. Heparinization and careful

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filtering cannot ensure that thrombosis or embolism will not occur. In our hands, blood commonly causes vasoconstriction which can block a perfusion entirely. Finally, as rupture of the red cells occurs, the electrolyte environment is progressively disturbed.

At the other extreme is Ringer-Locke's solution. This is known to sustain tissues in perfusion, but its oxygen-carrying capacity is limited to that of physical solution and it will rapidly cross the capillary wall and result in edema. From our point of view, furthermore, the presence of protein is necessary because steroid hormones are not soluble without it.

Following well-described techniques from the literature[2, 6] we have prepared relatively pure hemoglobin from outdated bank blood in 20 L. batches.

The material is stable at icebox temperature for a long time. The batch prepared in July, 1958, had a 94 per cent oxygen-carrying capacity on Jan. 5, 1960. The molecular size of hemoglobin approximates that of serum albumin so that a 6 per cent solution has a colloid osmotic pressure corresponding to that of plasma as well as an oxygen-carrying capacity half that of normal blood.

TABLE I. IN VIVO DIALYSIS OF HEMOGLOBIN IN RABBIT PERITONEUM

	BEFORE	AFTER
Na	156	140
	74	130
Cl	114	106
	54	99
K	2.5	4.1
	0.8	4.0
Ca	2.4	4.2
P	5.1	3.7
Mg	3.1	3.1
Hgb	6.35	6.0

O_2 capacity not changed.

The electrolyte composition of this preparation is the result of treating the laked cells with ion-exchange resin to remove the excess potassium. The deficiencies can be made up by the addition of the missing salts. Finding that the electrolyte values obtained from adding calculated amounts of salts often unexpectedly failed to attain the desired result, and hoping, perhaps a little mystically, to make the medium more "physiologic," we have tried in vivo dialysis of the hemoglobin by introducing as much as 500 ml. at a time into the peritoneal cavity of rabbits weighing 3 kilograms. If it is recovered by laparotomy from the heparinized animal in less than 2 hours it can be readily resterilized by Seitz filtration, has a relatively normal electrolyte composition, and retains its capacity to combine with oxygen. Table I shows the composition of the hemoglobin before and after in vivo dialysis.

METHOD

For perfusion, human ovaries are resected in such a manner as to preserve a long pedicle without injury to the vessels close to the gland. It seems best to leave the clamps on these and to immerse them at once in ice-cold saline containing heparin. (Liquid heparin must be

used cautiously; it contains phenol or benzyl alcohol which are toxic.) The clamps are cut away at once with a sharp blade under the fluid, which provides anticoagulant and prevents air embolism.

The ovarian arteries are attached to the apparatus and flushed with prepared cold hemoglobin as soon as the glands reach the laboratory. The vessels are small, the veins usually being larger than the arteries, but thinner-walled, and usually not more than 3 of these vessels per ovary can be cannulated. The cannulas are polyvinyl plastic.* This material is thermolabile enough to be drawn out into tapering cannulas of varying size (Fig. 3, *16*).

Fig. 1 is intended to illustrate the injection of the vasculature surrounding a corpus luteum with cannulation of a single ovarian artery. The selection of vessels (arteries versus veins) has been amply tested with latex injections and sections.

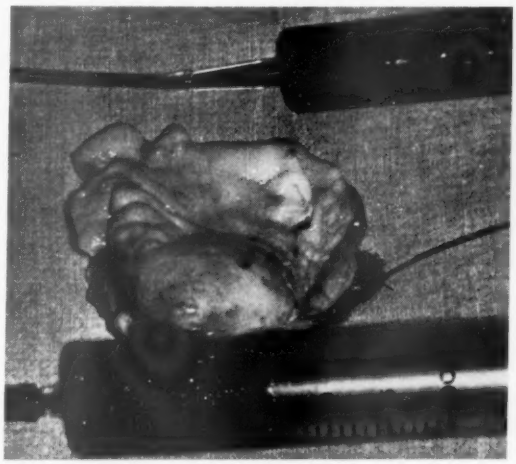


Fig. 1.

Microscopic slides (Fig. 2) taken at the end of perfusion illustrate vessels swept clear of red cells, the homogeneous material being hemoglobin.

Our apparatus (Fig. 3) permits the recycling of a small volume of perfusion medium. This small volume is important since it determines the frequency of contact of specific substrate, such as radioactive testosterone, with the gland tissue, and it also makes thorough extraction with organic solvent more practicable. The reservoir (Fig. 3, *12*), which is adapted from a rotary distilling apparatus (flash evaporator), is also an oxygenator in which the medium is kept cold by means of an ice bath. The 95 per cent O_2 and 5 per cent CO_2 mixture enters continuously through a polyvinyl catheter threaded through the lumen of the tube returning medium to the reservoir (Fig. 3, *11*).

The tubing from the reservoir cycles through a warm bath at 37° C. In this line a T tube and three-way stopcock (Fig. 3, *14*) allow a bubble to be injected for measurement of total flow through the pump.

The pump (Fig. 3, *1*) is of the eccentric rotor type.[8]† The stroke volume is 0.05 ml. The small-bore silicone rubber tubing (2.5 mm. outer diameter)‡ is able to tolerate prolonged pumping at high speeds. The speed of the pump is constant and reliable and can be varied over a wide range (10 to 400 r.p.m.) by means of a variable voltage DC amplifier. Beyond the pump is a bubble trap (Fig. 3, *3*) consisting of an inverted Y-tube with three-way stop-cock connection. Above this a short section of test tube serves as an air cushion (Fig. 3, *2*) by means of which pulsations are damped down to an amplitude smaller than 10 mm. Hg as checked by transductance manometer. The outlet head contains the tubes for ovarian cannulation, a connection for the mercury manometer (Fig. 3, *5*), and a bypass with a variable resistance

*Becton, Dickinson & Co., Rutherford, N. J., V 4044.
†Massey Dickinson Co., Watertown, Mass.
‡The Holter Co., Bridgeport, Pa.

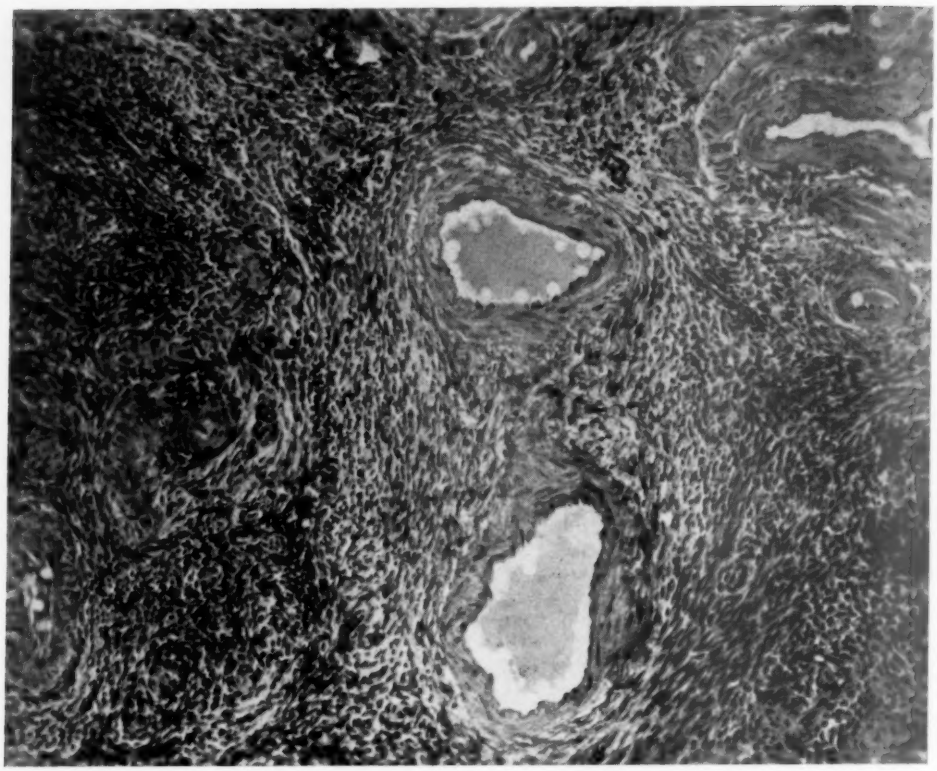
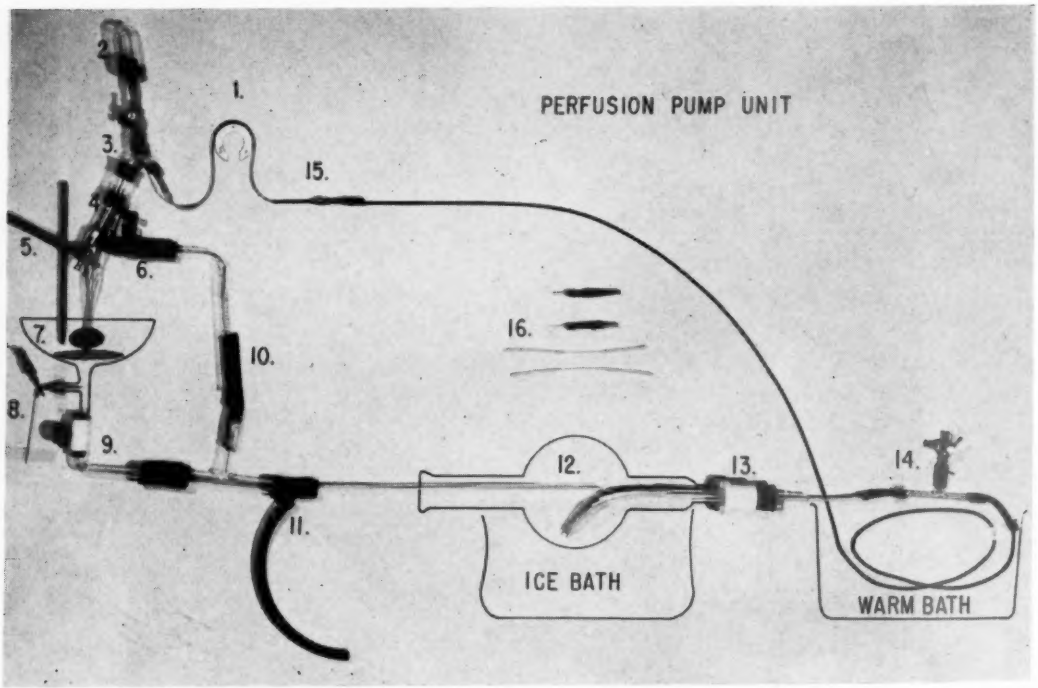


Fig. 2.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ICE BATH
WARM BATH

Fig. 3.

consisting of a small stainless steel needle valve (Hoke No. 823) (Fig. 3, *6*). The perfusion pressure can thus be regulated by this as well as by variation of the speed of the pump.

The bypass flow is measured by means of a Gaddum-type flowmeter (Fig. 3, *10*). The flow through the perfused organ is thus the total flow from the bubble measurement minus the bypass flow. With pump and bypass at a fixed setting, flow in the tissue is fairly constant within limits so that the resistance to flow in the tissue is roughly proportional to perfusion pressure.

The part of the apparatus which includes the silicone tubing, bubble trap, and cannulas (Fig. 3, *1-6, 15*) is mounted on a ringstand and filled with medium from a syringe while the glands are cannulated. The whole can then be transferred to the pump without danger of interruption of flow or introduction of air.

Two nylon filters (from disposable transfusion sets) are included in the circuit, one covering the reservoir outlet cannula (Fig. 3, *12*) and the other just beyond the pump (Fig. 3, *3*). These are important in preventing embolization by fragments of tissue.

The flask for the gland (Fig. 3, 7) has in its outlet a sidearm (Fig. 3, *8*) through which samples can be drawn. A needle goes through the rubber stopper on the sidearm to its junction with the stem. A second long No. 18 needle is attached to it on the outside with a section of silicone tubing which is closed with a small bulldog clamp. During perfusion the whole preparation is covered with a thick, insulating layer of sterile mineral oil. For sampling oxygen saturation, the needle-valve controlling the outlet of the apparatus (Fig. 3, *9*) is adjusted so as to bring the oil-hemoglobin interface to a level just above the sampling needle. (The stainless steel needle valve has proved more useful here also than the type illustrated.) Introduction of air is avoided by introduction of the long No. 18 needle into the bottom of the cuvette (capacity 0.1 ml.) which is then filled by gravity from below upward, 0.2 ml. being discarded by overflow. Oxygen saturation is then determined photometrically by the method of Gordy and Drabkin.[1]

It will be seen that on the whole the apparatus is made of inexpensive, easily replaceable parts. It is also autoclavable, polyvinyl, or Teflon being used rather than polyethylene. These factors are important since bacterial action or radioactive product contamination from a previous experiment could produce spurious results in experiments with radioactive steroids.

RESULTS

Rabbit Kidney Perfusion.—This organ was chosen because its size approximated that of the human ovary, but it has the advantage of possessing a simple pedicle with a single artery and vein so that the problem of leakage from arterial collaterals contributing to the outflow is removed. In early tests in which flows were grossly inadequate (averaging 0.05 ml. per gram per minute) in 13 determinations in 3 experiments, the oxygen-carrying capacity of the venous hemoglobin averaged 6.5 volumes per cent (range 5.4 to 7.0), the oxygen content average 3.1 volumes per cent (range 1.4 to 5.0) or an average difference of 3.4 volumes per cent. In the longest experiment the difference determined at 6 hours was 4.9 volumes per cent. More recent modifications have given more adequate flows, as shown in Table II. After the second hour, flow decreases as pressure rises. The almost threefold increase of arteriovenous oxygen difference does not compensate fully for this and there is some rise in lactate production. (The error in glucose determinations in our hands has been too large for these to be informative.) Here again, as in the earlier instances, the tissue is still biochemically alive although impaired. Urine production continues only during this earlier period. The urine hemoglobin was higher than that of the perfusate, 7.0 versus 5.4 Gm. per cent.

TABLE II. RABBIT KIDNEY PERFUSION

Time	12:20	1:40	2:30	4:10
Kidney weight (Gm.)	13.6	12.2	12.9	12.8
Pressure (cm. H_2O)	30	45	110	135
Flow (ml./min.)	6.0	4.6	4.0	0.4
A-V O_2 (vol. %)	–	–	0.85	2.45
A-V lactate (mg. %)	–	–6.4	–	–9.1

Cardiac Perfusion.—Unlike the kidney, perfusion of the mammalian heart by the Langendorff method[3, 5] with protein-free medium containing dissolved oxygen and glucose has long been known to be possible with maintenance of cardiac action, although the preparation does not survive long if output work is required.[3] This is a useful preparation on which to test a perfusion method, since the behavior of the heart gives a minute-by-minute record of what is happening. We have found that changing over to the hemoglobin medium from a medium containing balanced salts and glucose alone (Difco medium No. 199) or the same plus protein (fresh-frozen horse serum) after the heart has failed can restore cardiac action. In the perfusion of the heart of a 4 kilogram rabbit, the atria and right ventricle contracted without load, while the left ventricle, filled through the thebesian veins, contracted isometrically. Perfusion was started at 12:00 P.M. Failure of the left ventricle occurred first at about 3 hours and was followed by cardiac inaction at 4:00 P.M. Perfusion pressure had risen from 40 to 70 mm. Hg, lactate arteriovenous difference had risen from a small positive value to –4.4 mg. per cent. Hemoglobin was introduced at 70 mm. Hg at 4:30 P.M. and contraction of the heart resumed with the exception of the edematous left ventricle, despite oxygen embolism in making the transition. Opening of the left ventricle was followed by a feeble contraction here also. The arteriovenous lactate difference again fell with the institution of hemoglobin perfusion and was –0.4 mg. at the end of the experiment.

TABLE III. HUMAN OVARIAN PERFUSION

Time	11:00	12:20	1:20	2:20	4:00
Pressure (mm. Hg)	65	48	61	62	60
Flow (ml./min.)	–	–	–	11	12
A-V O_2 (vol. %)	–	0.5	0.4	0.6	0.3
A-V lactate (mg. %)	+2.1	+22.1	–8.6	–0.1	–2.1

Ovarian Perfusion.—Table III shows the protocol of a human ovary perfused at 28° C. The weight of the tissue was approximately 15 Gm. The flow includes leakage through collaterals. This is reflected by the small arteriovenous differences. Of chief importance is the constancy of perfusion pressure, the fact that the tissue in this instance did not become edematous, and, from the steroid viewpoint, the fact that the corpus luteum was perfused as confirmed by observation of the "bleeding" of a small nick in it. It is perhaps helpful that the ovary is a sturdier tissue than kidney or heart as judged by its well-known transplantability.

The final criterion of ovarian viability is steroid synthesis. We have obtained estradiol 17β from testosterone, 10 per cent of the recovered radioactivity being in the form of the former compound.[7] We have not obtained

estradiol 17β except as a metabolite of testosterone, that is, the specific radioactivity of the recovered estradiol in the absence of carrier is close to that of the original testosterone. Our chief concern at present is the pursuit of other still unidentified ovarian products from tagged testosterone.

DISCUSSION

Ideally, the adequacy of a perfusion should be determined by a measurement of work performed by the perfused tissue. This is not feasible by any simple means except where mechanical work can be measured. A second-best measure is of energy consumption by the tissue. Oxygen consumption and lactate production account for the major part of this. This in turn should ideally be referred to some measure such as tissue weight and then compared with a known normal value. Better still, the proportion of tissue actually perfused at a given time should be known, for example, by determination of antipyrine or sucrose space.

For our present purposes qualitative comparisons seem sufficient. It suffices for the moment that a particular preparation seems to be a good enough perfusion to justify the elaborate study necessary for steroid identification.

We know that our present hemoglobin medium is not fully adequate. We suspect that the best medium would be hemoglobin dialyzed in vitro against serum with a small amount of serum added to it. The problem of carrying this out on a bulk preparation, however, has still to be met.

SUMMARY

1. A simple method of perfusion, applicable to a whole organ or to a small part of an organ, is described.

2. Evidence is brought forth to show that tissue survives under the conditions provided.

3. The requirements and limitations of the method with respect to the study of steroid production by the human ovary are briefly discussed.

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REFERENCES

1. Gordy, E., and Drabkin, D. L.: Spectrophotometric Studies. XVI. Determination of Oxygen Saturation of Blood by a Simplified Technique, Applicable to Standard Equipment, J. Biol. Chem. **227**: 285, 1957.
2. Hamilton, P. B., Farr, E. L., Hiller, A., and Van Slyke, D. D.: Preparation of Hemoglobin Solutions for Intravenous Infusions, J. Exper. Med. **86**: 456, 1947.
3. Langendorff, O.: Untersuchungen am überlebenden Saügethierherzen, Arch. gesammte physiol. **61**: 291, 1895.
4. Locke, F. S., and Rosenheim, O.: Contributions to the Physiology of the Isolated Heart, J. Physiol. **36**: 205, 1907.
5. Martin, N.: Studies From the Biology Laboratory, Baltimore, 1881, Johns Hopkins University, vol. II, p. 119.
6. Renkin, E. M., and Zaun, B. D.: Effects of Adrenal Hormones on Capillary Permeability in Perfused Rat Tissues, Am. J. Physiol. **180**: 498, 1955.
7. Richardson, G. S., Ulfelder, H., and Vineze, L.: Steroid Metabolism of the Human Ovary Under Perfusion With Hemoglobin Solution, S. Forum **9**: 658-663, 1959.
8. Shaw, R. S., and Grove-Rasmussen, M.: Rotary Transfusion Pump—Studies on Hemolysis, Ann. Surg. **138**: 928, 1953.

THE INDUCTION OF TUMOR IMMUNITY WITH A NEW FILTERABLE AGENT

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PROPHYLACTIC and destructive tumor immunity against transplantable tumors has been produced by various immunologic means. It is not our purpose to discuss comprehensively the diverse and numerous studies demonstrating the production of immunity to tumors. Selected examples of experimental induction of tumor immunity, based on different levels of specificity, will be briefly cited.

In conditions where the immunized animal is genetically dissimilar from the tumor and its natural host, immunity to tumors has been induced with both neoplastic and non-neoplastic tissues, as well as fractions and extracts thereof. Barrett[1] immunized BALB/cN mice against sarcoma 49, a DBA tumor, by using DBA/2N red cells or stroma as the antigen. Successful resistance against tumors has been achieved by immunization with viable tumor cells that grow progressively in the treated animal[7, 11, 13, 21, 24, 27, 28] and with tumors that do not grow progressively in treated hosts.[6, 10, 22, 23, 26, 32]

Subcellular particles, as well as extracts and fractions of tumor cells, have been used as antigens to produce heterologous antibodies which are toxic to the tumor cells.[3-5, 14, 18] Imagawa and co-workers[16] demonstrated cytotoxic activity against mammary gland carcinomas induced by and containing Bittner's milk agent. Heterologous antisera prepared by immunization with the Bittner virus extracted from both normal and cancerous mammary glands produced lethal changes in mammary gland tumor cells. Law and Malmgren,[20] however, reported negative results in similar experiments. Burmester[2] has been able to produce a homologous antisera, cytotoxic for avian lymphoid tumors, by immunizing chickens with fractions of the disrupted tumor cells containing the causative virus. It should be strongly emphasized that in every instance where tumor immunity has been induced, the immunizing antigens have been whole tumor cells or fractions thereof, normal tissues from a strain genetically different from the immunized strain but identical or closely related genetically to the tumor tested, or fractions of tumor cells containing the virus etiological for the tumor tested.

The present communication reports the induction of prophylactic and destructive immunity to several widely employed transplantable rat tumors by a new filterable replicating agent. The demonstration of the tumor immunity

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induced by this agent has been accomplished by 3 well-accepted methods for demonstrating the effects of humoral antibody. These techniques include the production of total resistance to the transplantation of ordinarily easily transplantable standard tumors, the production of cytomorphologic toxic changes in cancer cells exposed to this immune serum, and the neutralization or loss of viability of tumor cells incubated in immune serum. The results to be reported in this article demonstrate the production of immunity to neoplastic cells by something other than tumor cells or fractions thereof, and suggest that the 6 tumors studied share a common antigen not possessed by normal rat tissues.

MATERIALS AND METHODS

Nature of Immunizing Agent.—The immunizing material employed throughout these studies is an infectious replicating agent discovered in our laboratories and responsible for an acute infectious hemolytic anemia.[29, 30] At the date of this writing, the agent has been transmitted serially in over 140 passages in some 2,500 rats and has remained stable both in its infectivity and in the hemolytic syndrome it produces. Transmission of the hemolytic disease to normal animals is effected by the inoculation of any of a variety of tissues or fluids taken from an animal at the height of the disease. The agent is present in good titer in sera or blood from animals at the height of the hemolytic syndrome, with 1/100,000 ml. of serum infecting normal animals upon intraperitoneal administration. The agent (which we abbreviate FHA, filterable hemolytic anemia) is extremely small in size, being filterable through Seitz S-1 filters with pore diameters of 0.02 μ and being nonsedimentable in the ultracentrifuge even under such drastic conditions as 144,000 g for 10 or 12 hours.

In addition to the induction of tumor immunity, which will be described, infection with FHA results in numerous biologic manifestations. Following the inoculation of infectious FHA agent to a normal rat there is an incubation period of 2½ to 12 days depending on the dosage of the inoculum. Then acute intravascular hemolysis occurs precipitously within a 24-hour period or less, with a drop of blood hemoglobin to 2 to 4 Gm. per cent. Two weeks to 6 months after the recovery from infection, there appears in a small percentage of animals a variety of late lesions, the majority of which are imperfectly understood.[29] The agent is strongly antigenic, stimulating the production of neutralizing antibodies. Sera from animals that have recovered from infection with FHA not only neutralize the agent in vitro but also confer passive immunity to infection with FHA in normal animals.

Experimental Animals.—Pen-bred, young adult, virginal, female, Sprague-Dawley, Holtzman rats were used throughout. Weights of animals ranged between 160 to 200 grams at the initiation of the experiment. FHA-immune animals were animals which had recovered from infection with FHA (infection was effected by intraperitoneal inoculation of 0.05 to 1 ml. of infectious whole blood). Repeated inoculations of the FHA animals varied with the experiment, animals receiving none or up to 2 subsequent intraperitoneal inoculations of 1 or 0.5 ml. of infectious whole blood. The inoculation of FHA agent to recovered animals was entirely without gross symptomatic effects. In addition, 3 groups of animals also received 3 subcutaneous injections of FHA infectious whole blood mixed with Freund's adjuvant. Control animals were animals from the same shipment that received, in the initial experiments, 1 c.c. of whole normal blood intraperitoneally on 1, 2, or 3 occasions. It was noted that the inoculation of normal animals with normal blood did not influence the incidence of tumor "takes" or deaths. Therefore, in the later experiments control animals were not administered normal blood.

Tumors and Transplantation.—It was found early in the course of experiments with FHA that standard transplantable rat tumors, in our laboratories, could not be maintained

free of the infectious FHA agent despite transplantation under rigorously aseptic techniques. This was not a matter of simple contamination only, for administration of normal blood or normal tissue brei did not result in occurrence of the hemolytic anemia syndrome. Because of this contamination some of the tumors have been maintained in an adjacent building which is completely physically isolated from the housing of the laboratory where the experiments were performed. Two classes of tumors were therefore used in these experiments, tumors unassociated with the FHA agent and tumors which transmit or carry the agent. In the former category are the Walker 256 carcinosarcoma FHA-free, Novikoff hepatoma, Murphy-Sturm lymphosarcoma, and a spontaneous fibrosarcoma which originated in our laboratories. Tumors in the latter category, that is, tumors which are associated with and transmit the FHA agent, are the Walker 256 carcinosarcoma with the FHA agent (the same tumor as the FHA-free Walker tumor except the latter is maintained in a separate building and has remained FHA-free), the Miller hepatoma, and the Flexner-Jobling carcinoma. Both Walker tumors, Miller hepatoma, and Novikoff hepatoma have resulted in 100 per cent tumor takes with progression to death in many hundreds of transplantations in normal animals with cell dosages in the range employed in these studies. The 2 strains of the Walker 256 carcinosarcoma, Miller hepatoma, and Novikoff hepatoma are all maintained in the ascitic form and were transplanted by employing appropriate dilutions with normal saline solution or Hanks'[12] buffered salt solution of the freshly centrifuged ascites cells. The fibrosarcoma, Murphy-Sturm lymphosarcoma, and Flexner-Jobling carcinoma are maintained in the solid form and were transplanted by employing tumor cell suspensions prepared by excision of tumor fragments uninvolved with hemorrhage or necrosis which were diluted to the proper extent and homogenized in a very loose-fitting Potter-Elvejhem type glass homogenizer. Cell counts were performed with standard white blood cell diluting pipettes and a hemocytometer. The diluent was 2 per cent acetic acid. Tumor cell dosage was identical in the control animals and the FHA-immune animals for any experiment, but tumor cell dosage varied from one experiment to another. Transplantation of the tumor cell suspensions was intraperitoneal through a 20-gauge needle. Tumors were transplanted into FHA-immune animals 16 to 101 days after the acute hemolytic crisis due to infection with FHA, and from 6 to 54 days after the last injection of FHA-infectious blood. However, in any one experiment all tumor transplantations were performed on the same day, employing the same tumor cell suspension for the control as for the immune animals. Volume and cell dosage were kept constant within an experiment.

Tumor Incidence, Takes, and Deaths.—Animals in all experiments were checked and palpated daily for tumor takes and deaths. Upon the death or sacrifice of an animal a thorough postmortem examination was performed and any area obviously or suspiciously involved with tumor was removed for histologic examination. Such tissue was fixed in 10 per cent formalin and stained with hematoxylin and eosin. Animals that died from ascitic tumors invariably had solid tumor nodules as well. These nodules rather than the ascitic fluid were selected for histologic examination.

Cytomorphologic Studies.—Three tumors were studied in these experiments: the Walker 256 carcinosarcoma FHA-free, the Miller hepatoma, and the Flexner-Jobling carcinoma. Ascites tumor was removed asceptically from tumor-bearing animals and centrifuged at 2,000 r.p.m. for 5 minutes. The supernatant fluid was then decanted, 9 volumes of Hanks' buffered salt solution was added, and the tumor cells were suspended in it. The tumor cell suspension was then loaded in a visualization chamber which consisted of a rectangular sheet of stainless steel with a thickness of 0.9 mm., and with a circular punched-out hole 35 mm. in diameter on which were cemented with a melted mixture of beeswax and paraffin two 50 by 50 mm. No. 1 cover glasses. On one side, the bottom, the cover glass completely covered the circular hole. On the other side, the cover glass was applied in such a manner that there was a small area not covered with glass through which fluid could be placed into the chamber or withdrawn at will through a 25-gauge needle. This small hole was sealed with the melted beeswax paraffin cement prior to microscopic observation of the cells. After the tumor cell suspension had been placed in the chamber, it was immediately

inverted and allowed to rest at room temperature for 10 minutes so that the cells in the suspension would layer out and adhere on the glass surface. After 10 minutes the chamber was righted and the fluid withdrawn. A great many cells remained behind, adherent to the bottom surface of the top cover slip as a result of layering-out during the inversion. The withdrawn fluid was then replaced with the serum to be tested. FHA-immune sera were sera from animals recovered from infection with FHA agent which had been inoculated on 2 subsequent occasions with 1 ml. of FHA infectious whole blood. Normal sera were from animals which were shipment mates but which had received no injections, or from animals which had received normal whole blood. The chamber was then sealed and observation commenced. Observation was carried out with the chamber on the stage of a Zeiss phase contrast microscope which was completely enclosed in a constant temperature box maintained at 37° C. Magnification was times 800. Time lapse cinemicrography was instituted when a suitable field was found. Visualization by means of a prism and a second ocular was possible throughout the period of photography. Observation was continued over a 2-hour period. Recordak Micro-File 16 mm. film was used. Forty-eight frames per minute were exposed. Fig. 2 (*1-16*) was made by enlargement of single frames.

Neutralization Test.—A neutralization test was performed on the Flexner-Jobling carcinoma. Tumorous ascitic fluid was centrifuged under the conditions specified above, suspended in Hanks' buffered salt solution, and counted. The cell suspension was then diluted to the proper volume, and aliquots of this suspension were mixed with FHA-immune serum and with normal rat serum. Complement (fresh, normal, undiluted guinea pig serum) was added to each in identical dosage from the same guinea pig. Incubation was then carried out at 25° C. for 4 hours with occasional mixing. Aliquots from the resistant serum-treated cells were then injected into each of 10 normal rats and aliquots of the normal serum-treated cells were injected into each of 9 normal animals. The animals were followed for tumor takes and deaths.

Levels of Significance.—In experiments involving tumor takes and or deaths as parameters, 2 by 2 chi-square tests of contingency with Yates' correction for continuity were performed to determine levels of significance between FHA-immune and control animals. In the one experiment with the Novikoff hepatoma, where survival data are compared between the FHA-immune group and the control animals, 1 factor analysis of variance with 2 groups was calculated. Variances were made homogeneous by reciprocal transformation before analysis.

RESULTS

Studies of Active Resistance to Transplantation of Tumors.—As stated previously, 2 classes of tumors were tested. One category included tumors which are free of the FHA agent, that is, on transplantation into a normal animal the tumor itself does not produce the hemolytic anemia. The second category includes tumors that induce the hemolytic anemia syndrome in a variable percentage of animals with transplants. Whether the FHA agent is associated intimately with the tumor cells or with contaminant leukocytes or other cells is a matter of conjecture. We shall consider the first category first.

Two separate experiments were performed with the Novikoff hepatoma. In the first experiment 9 FHA-immune animals and 9 control animals were administered 1.2 million hepatoma cells (Table I). In this experiment 2 animals in the FHA-immune group did not develop tumors whereas all 9 of the control animals developed and died of the tumor. In experiment I, therefore, there was 22 per cent complete protection from the transplantation of the Novikoff hepatoma in the FHA-immune animals. Animals in this experiment were followed for 67 days after tumor transplantation or 51 days after the last

death in the control group. At this time the 2 living FHA-immune animals were sacrificed and found to be tumor-free. In experiment II, 15 FHA-immune animals and 15 control animals were administered 500,000 hepatoma cells. This experiment is still in progress so the final results cannot be tabulated. However, up to the present time, all of the control animals have developed tumors and all but 1 have died. It is most unlikely that any other than the 4 FHA-immune animals that have already developed tumors will develop tumors at a later date, for it was found in experiment I, and in experiments with other tumors, that although survival time in FHA-immune animals which develop tumors may be considerably prolonged, the latent period, or time for the first appearance of the tumor in transplanted animals, is not prolonged.

TABLE I. NOVIKOFF HEPATOMA

		NO. OF RATS	TUMOR TAKES AND DEATHS	LIVING WITH TUMOR	SURVIVAL AFTER TRANSPLANTATION (DAYS)	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	I	9	7	0	31.6 (7 rats)	2	22.2
	II	15	0	4	—	11	73.3
		24	7	4		13	54.2
Controls	I	9	9	0	11.9	0	0
	II	15	14	1	—	0	0
		24	23	1		0	0

FHA-immune animals, experiments I and II, received 2 FHA inoculations following recovery from original infection and those of experiment I also received 2 FHA inoculations with Freund's adjuvant. Animals in experiment I received 1.2 million cells and those in experiment II 500,000 cells. In the 2 experiments, FHA-immune animals were 101 and 16 days after acute hemolytic crisis and 36 and 6 days after the last FHA inoculation for the first and second experiments, respectively, on the day of the tumor transplantation. $\chi^2 = 15.1$; $p < 0.001$.

In the first experiment the control animals received no normal blood, and in the second experiment, the control animals received 3 1 ml. inoculations of normal blood on the same dates that the FHA-immune animals received the immunizing dosage of FHA. It is readily apparent that immunization with normal blood to control animals did not influence the incidence or course of tumor transplants. The combined values from experiments I and II with Novikoff hepatoma reveal that 13 of the 24 FHA-immune animals were totally protected from transplantation of the tumor whereas there were 100 per cent tumor takes in the controls. These differences are statistically significant. The discrepancy of total resistance rates in FHA-immune animals of 22 per cent in the first experiment and of 73 per cent in experiment II is not readily explicable. As indicated in the legend of Table I, tumor transplantation was performed 101 days after the acute hemolytic crisis in FHA-immune animals in experiment I and 36 days after the last FHA inoculation, whereas the animals in experiment II were only 16 days convalescent from infection with acute hemolytic anemia and it was 6 days after the last FHA inoculation on the day of the tumor transplant. However, the animals in experiment I received 1.2 million hepatoma cells and those in experiment II received 500,000 cells.

The 7 animals in the FHA-immune group which did develop tumors survived a considerably longer period of time than did the control animals. Thus,

the FHA-immune animals with tumors survived a mean of 31.6 days following transplantation of the Novikoff hepatoma, whereas the control animals lived only 11.9 days. The cumulative mortality data are plotted in Fig. 1. The difference in survival time is significant statistically with a p value of less than 0.005.

TABLE II. MURPHY-STURM LYMPHOSARCOMA

	NO. OF RATS	TUMOR DEATHS	LIVING AND TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	15	2	13	86.7
Control	16	14	2	12.5

FHA-immune animals received 2 FHA inoculations following recovery from original infection. Animals were transplanted with 2 million cells. FHA-immune animals were 97 days after acute hemolytic crisis and 16 days after last FHA injection on the day of transplantation. $\chi^2 = 14.2$; $p < 0.001$.

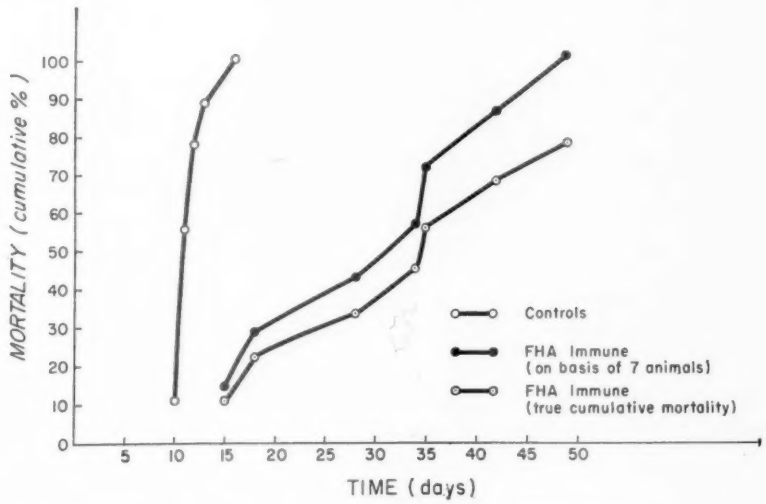

Fig. 1.—Survival time after transplantation of Novikoff hepatoma in FHA-immune and control animals. Plotted as cumulative mortality. Immune mortality graphed both on basis of the 7 animals which had a tumor take and as true mortality of the total 9 animals.

The Murphy-Sturm lymphosarcoma (Table II) was transplanted into 15 FHA-immune and 16 control animals. There were 2 tumor deaths in the FHA-immune group, 13 animals now living and tumor free, corresponding to 87 per cent tumor-free animals. Fourteen of the 16 control animals died of the tumor, 2 rats are now living and tumor-free for a 12.5 per cent incidence of tumor-free animals. This experiment is now in its forty-ninth day. The differences enumerated above are statistically significant. The results of this experiment attain added significance upon consideration that the transplantation into the FHA-immune and the control animals was the first transplant this tumor had undergone in our laboratory and, therefore, minimal opportunity for contamination with the FHA agent was available. Included in the legend of Table II and the other tables in this section are data furnishing information on the number of immunizations with FHA, the use or non-use of Freund's adjuvant, tumor cell dosage and the time following the acute hemolytic crises as well as the time following the last inoculation with FHA that the animals were transplanted with tumors.

Nine FHA-immune animals and 9 control animals were transplanted with the FHA agent-free Walker 256 carcinosarcoma (Table III). There were 5 tumor takes in the immune group, one of which was a tumor death, and 9 tumor takes and deaths in the control group. Four FHA-immune animals remained totally tumor-free for a total resistance of 44 per cent. Four of the 5 tumors in the immune group were of an unusual variety. Only 1 of the 5 was a typical Walker tumor. The atypical tumors were characterized by an unusual growth behavior pattern. Ascites was not formed in any of the 4 animals bearing the modified tumors. Furthermore, each of these 4 animals had only 1 tumor nodule and that 1 nodule was in an abnormal location in each instance.

TABLE III. WALKER 256 CARCINOSARCOMA FHA-FREE

	NO. OF RATS	TUMOR TAKES	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	9	5*	4	44.4
Control	9	9	0	0

FHA-immune animals received 2 FHA inoculations and 2 FHA inoculations with Freund's adjuvant following recovery from original infection. Animals were transplanted with 5.3 million cells. FHA-immune animals were 85 days after acute hemolytic crisis and 11 days after last FHA injection on day of transplantation. $\chi^2 = 2.8$; $p < 0.1$.

*Four modified tumors, 1 characteristic tumor.

Two of the animals had the nodule in the ovary, 1 in the gastrohepatic omentum, and 1 in the mesentery of the small bowel. In control animals, the Walker tumor always produces ascites, forms numerous solid nodules, routinely produces a nodule in the tract of the needle used for transplanting the tumor, and these solid tumor nodules characteristically infiltrate and invade surrounding tissues and structures, rather than being encapsulated as the modified tumors were. The growth of the modified tumors was not progressive but rather they appeared to reach a constant size and to remain at that size. None of these animals died; they were all sacrificed. Histologically, these tumors displayed atypical characteristics also. There was extensive necrosis in all of them with the mass of gross tumor tissue estimated to consist of between 70 and 90 per cent of necrotic material and only a small amount of viable tumor cells. The viable tissue that was present histologically was unusual, characterized by the presence of fibrous tissue septa separating the tumor into fascicles of viable cells, more differentiated appearance of the tumor, and lack of any obvious carcinomatous areas, and composed entirely of tissue that resembled a fascicular sarcoma. Because these animals were sacrificed, it is impossible to state what the natural history or outcome would have been. The differences in the incidence of tumor-free animals in the FHA-immune group and control group although 44 and 0 per cent, respectively, are not significant because of the small sample size ($p < 0.1$). However, our experiences with this line of the Walker 256 carcinosarcoma have been that the transplantation intra-abdominally of tumor cells in the dosage range used in the experiment has resulted in 100 per cent incidence of tumor takes and deaths in many other normal animals. If this be taken as the true incidence, the deviation from 0 to 44 per cent becomes extremely significant.

The last experiment with tumors not transmitting the FHA agent was with the fibrosarcoma. Ten animals in each group, FHA-immune and controls, underwent transplantation. The results listed in Table IV indicate that 1 animal in the FHA-immune group died of tumor and 3 are now living with tumors. In the control group, 5 animals have died from the tumor and 2 are living with tumors. Sixty per cent of the FHA-immune animals are tumor-free and 30 per cent of the control animals are tumor-free. The low degree of immunity obtained in this experiment might be a reflection of 2 factors. The animals transplanted with the fibrosarcoma in the immune group had recovered from infection with FHA but had not received any subsequent inoculations with the agent. Second, the FHA-immune animals were 57 days after acute hemolytic crisis on the day of the tumor transplant. The differences observed in this experiment are not significant statistically.

TABLE IV. FIBROSARCOMA

	NO. OF RATS	TUMOR DEATHS	LIVING BUT WITH TUMOR	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	10	1	3	6	60
Controls	10	5	2	3	30

FHA-immune animals received no inoculations of FHA following recovery from the original infection. Animals were transplanted with 1.2 million cells. FHA-immune animals were 57 days after acute hemolytic crisis on day of tumor transplantation. $\chi^2 = 0.81$; not significant.

The next group of experiments was performed with tumors that carry the FHA agent. Three different experiments were carried out with the Walker 256 carcinosarcoma associated with FHA (Table V). In all 3 experiments, all control animals developed and died of the tumors. The FHA-immune animals were protected from tumor transplantation. In the first experiment there was a 50 per cent incidence of total protection, in the second 15 per cent, and the third 22 per cent, for an average of 25 per cent. The surviving FHA-immune animals were sacrificed, examined, and found tumor-free 66 to 102 days after the last control animal death. The differences between FHA-immune and control animals are significant statistically. As in the case of the Novikoff hepatoma, the injection of normal blood to the control animals of experiments I and II did not influence the tumor takes. The greatest difference in tumor takes in the experimental groups were noted between FHA-immune animals in experiment I and those in experiment II. These 2 groups were apparently treated quite similarly. The reason for this discrepancy is obscure. Quite possibly it represents nothing more than a sampling error.

Experiments with the Miller hepatoma (Table VI) showed that the control animals all developed tumors and died. More than 17 control animals underwent transplantation. However, since the Miller hepatoma carried the FHA in a virulent form some of the animals transplanted with the Miller hepatoma died 4 and 5 days after tumor transplantation from acute hemolytic anemia. These animals are not counted as tumor deaths. Only those animals which had not died from the effects of acute hemolytic anemia are included as definitive animals. Of the 9 FHA-immune animals transplanted with the Miller tumor, 8 remained totally tumor-free for a protection of 89 per cent. All

TABLE V. WALKER 256 CARCINOSARCOMA WITH FHA AGENT

		NO. OF RATS	TUMOR TAKES AND DEATHS	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	I	6	3	3	50
	II	13	11	2	15.4
	III	9	7	2	22.2
		28	21	7	25
Control	I	6	6	0	0
	II	7	7	0	0
	III	9	9	0	0
		22	22	0	0

FHA-immune animal experiments I, II, and III received 1, 2, and 2 FHA inoculations following recovery from original infection and those of experiment III received 2 FHA inoculations with Freund's adjuvant. Animals in experiment I received 40 million tumor cells, experiment II 35 million, experiment III 190,000. FHA-immune animals were 10 days after acute hemolytic crisis in experiment I, 11 days in experiment II, and 81 days in experiment III and 4, 5 and 10 days after last FHA inoculations, respectively, for the 3 experiments on the day of the tumor transplantation. $\chi^2 = 4.49$; $p < 0.05$.

control animals died from the effects of the Miller tumor 7 to 19 days after transplantation. The 1 FHA-immune animal which developed a tumor died 64 days after transplantation. This animal was found to have a most unusual tumor. Although the tumor was transplanted intraperitoneally, no intraperitoneal tumor was found. Rather the entire thoracic cavity was largely replaced with a 3 cm. mass which, on gross observation as well as histologic confirmation, proved to be about 90 per cent necrosis. Ten per cent or less of the tumor mass was Miller tumor which, although encapsulated, had replaced a great deal of pulmonary tissue. This kind of growth pattern following transplantation of the Miller hepatoma intraperitoneally has never been observed by the authors in normal animals. The FHA-immune animals were sacrificed 86 days after the last control death and were tumor-free. The difference in tumor incidence between the 2 groups is significant.

TABLE VI. MILLER HEPATOMA

	NO. OF RATS	TUMOR TAKES AND DEATHS	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	9	1*	8	88.9
Control	17	17	0	0

FHA-immune animals received 2 FHA inoculations and 2 FHA inoculations with Freund's adjuvant following recovery from original infection. Animals were transplanted with 2.6 million cells. FHA-immune animals were 74 days after acute hemolytic crisis and 9 days after last FHA injection on day of transplantation. $\chi^2 = 17.9$; $p < 0.001$.

*Modified tumor.

Experiments with Flexner-Jobling carcinoma provided some rather unexpected findings. This tumor was transplanted for the first time in this laboratory into animals employed in the experiment listed under Table VII. Sixteen controls received transplants. Although the tumor was transplanted under aseptic conditions, 12 of the control animals developed symptoms of the acute hemolytic anemia 8 days after the tumor transplantation. The other 4 control animals did not. The 12 control animals which developed infectious anemia died within 1½ days after the onset of symptoms. These animals all had tumors. However, it is impossible to state whether the cause of death was the tumor or the infectious hemolytic anemia. The remaining 4 control animals lived for longer periods of time, and 2 of these animals developed

tumors and died of the tumors whereas 2 remained tumor-free. Thus a total of 14 out of the 16 controls had growing tumors at the time of death. Sixteen FHA-immune animals were inoculated at the same time. There were only 2 tumor deaths in this group. The other 14 remained asymptomatic. The 14 FHA-immune animals and the 2 control animals were sacrificed 50 days after the tumor transplantation. Both control animals were found to be tumor-free.

TABLE VII. FLEXNER-JOBLING

	NO. OF RATS	TUMOR AT DEATH	TUMORS AT TIME OF SACRIFICE	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	16	2	3*	11	68.8
Control	16	14†	0	2	12.5

FHA-immune animals received 2 FHA inoculations following recovery from original infection. Animals were transplanted with 1.2 million cells. FHA-immune animals were 85 days after acute hemolytic crisis and 11 days after last FHA injection on day of transplantation. $\chi^2 = 8.3$; $p < 0.01$.

*All 3 tumors—small, largely necrotic, encapsulaated, and surrounded by dense mononuclear infiltration.

†See text.

Eleven of the 14 FHA-immune animals were found to be tumor-free, but tumors were found in the 3 others. These 3 tumors were no larger than 3 mm. in diameter, completely encapsulated and surrounded by mononuclear cells, and all appeared microscopically, at least, not to be growing and were extensively necrotic. The interpretation of these results is difficult. It is quite likely that no tumors would have been found in the FHA-immune group had the animals been allowed to live for a longer period of time. Conversely, it is also quite likely that had the animals been sacrificed earlier a higher percentage of animals would have been found to contain nests of tumor cells. None the less, these 3 animals are included in the tumor group, leaving 69 per cent of the FHA-immune animals tumor-free and 12.5 per cent of the control animals. These differences again are significant statistically.

TABLE VIII. RESISTANCE TO TUMOR TRANSPLANTATION

	NO. OF RATS	TUMOR-FREE	TUMOR-FREE (PER CENT)
FHA-immune	111	62	55.9
Control	114	7	6.1

Combined results with all tumors. $\chi^2 = 63.2$; $p < 0.001$.

Table VIII is a summary of tumor transplantation studies in FHA-immune and control animals. In all, 111 FHA-immune animals were transplanted with the tumors listed before. Sixty remained entirely free of tumors for a total immunity incidence of 54 per cent. Of 114 control animals receiving transplants of the same tumors only 7 remained tumor-free which is equivalent to 6 per cent. These differences are highly significant statistically.

In Vitro Cytotoxicity Studies.—A total of 15 experiments were performed. In each experiment, tumor cells were incubated with serum from an animal that was FHA-immune and with serum from a control. Three different tumors were tested, the Walker 256 carcinosarcoma FHA-free, the Flexner-Jobling carcinoma, and the Miller hepatoma. Conditions of the experiment

were described. With 2 exceptions, the results were essentially identical with all 3 tumors. Two samples of FHA-immune serum which were tested against Miller hepatoma cells only were found to possess little cytotoxic activity. In all other instances, the 3 types of tumor cells incubated with resistant sera showed progressive cytotoxic changes. Tumor cell suspensions incubated with control rat sera displayed no cytotoxic changes.

The cytotoxicity of FHA serum for tumor cells was found to be quite specific. Frequently in the microscopic field there were tumor cells, side by side with normal cells (macrophages and leukocytes). It was found that while the sera from FHA-immune animals were extremely toxic for the cancer cells, the normal cells remained viable and showed no damage whatsoever for the entire observation period of 2 hours. The cytotoxic changes were retraction of normal cellular processes and pseudopods, a rounding up of the cell, increasing density of the nucleus, disappearance of the nucleolus, marked increase in size of cytoplasmic granulation with clumping of granules, vacuolization of the cytoplasm, and extrusion of the cytoplasm in the form of cytoplasmic "blisters" and "bubbles." A characteristic change associated with death of the cell that was most clearly observed on the photographic film was complete cessation of all cytoplasmic motion. Also of interest was the occurrence of progressive morphologic destruction after death of the cells. Eventually the cells lost all distinguishing features of an organized cytoarchitecture and were transformed into nothing more than a globular mass of granules. The cytotoxic changes observed are identical to the changes induced by homologous and heterologous cytotoxic immune serum.[6, 8, 17, 19, 25, 31, 34] The specificity of these immunologic changes was stressed by Kalfayan and Kidd[17] who could not induce similar changes with a variety of toxic stimuli including several lytic enzymes, heat, surface active agents, and hypotonic solutions. It should again be emphasized that although 2 samples of this FHA-immune serum were only questionably toxic to Miller hepatoma cells, none of the samples of normal rat serum showed toxicity. The cytotoxic changes described began 5 to 20 minutes after the addition of the FHA-immune serum. Fig. 2 illustrates the progressive changes occurring in a Flexner-Jobling carcinoma cell incubated with FHA-immune serum.

Neutralization.—An experiment was performed to determine the effect of FHA-immune serum on the viability of Flexner-Jobling tumor cells. In this experiment, 1 part of Flexner-Jobling tumor cell suspension was mixed with 2 parts of FHA-immune serum and 2 parts of complement (fresh, undiluted guinea pig serum). The control cells were an aliquot of 1 part cell suspension mixed with 2 parts of normal rat serum and 2 parts of the same source of complement. The concentration of tumor cells in the FHA serum mixture as well as in the control serum mixture was 2 million per milliliter. Both mixtures were incubated at 25° C. for 4 hours. At the end of the incubation period 0.2 ml. of the immune serum-cell mixture was inoculated into each of 10 normal rats and 0.2 ml. of the control serum-cell mixture was inoculated into each of another 9 normal rats. The dosage of cells to each animal was 560,000.

Table IX illustrates the destruction of the viability of tumor cells by the FHA-immune serum. None of the 10 animals inoculated with cells incubated with the immune serum-complement developed tumors, whereas 7 of the 9 animals receiving identical dosage of tumor cells incubated in control sera developed and died of the Flexner-Jobling tumor.

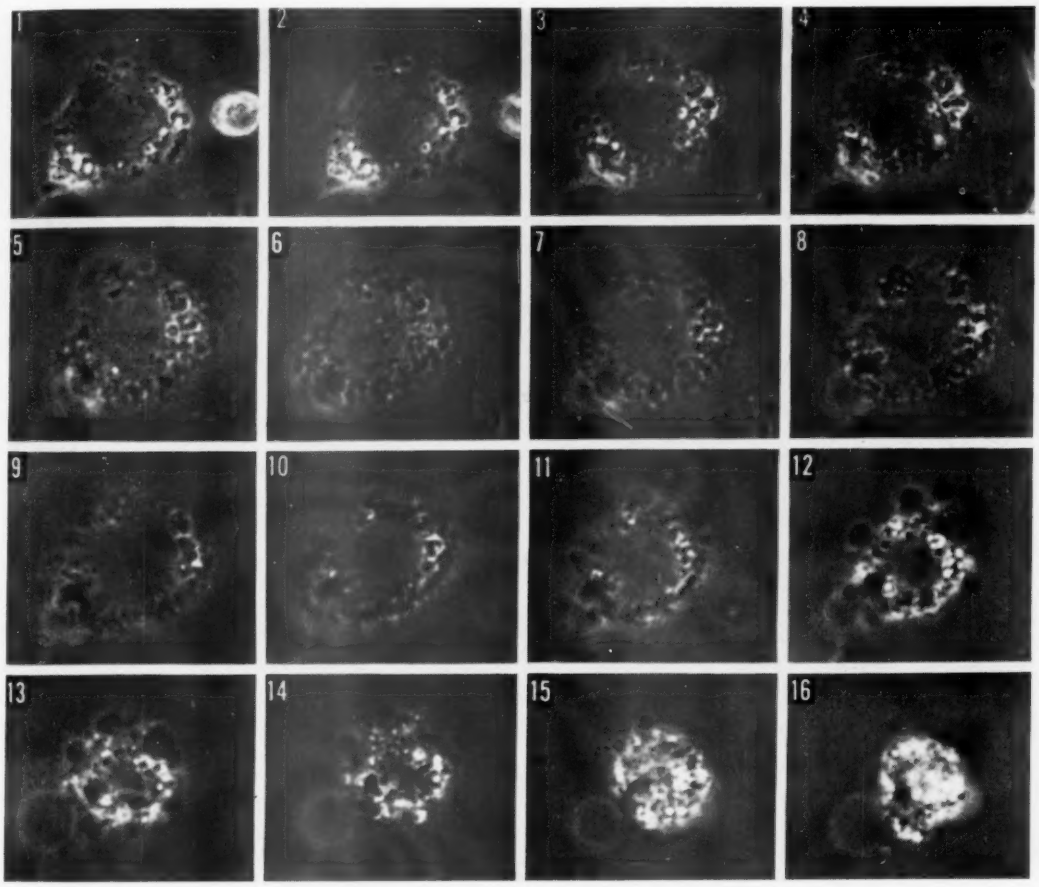

Fig. 2.—Effect of the in vitro incubation of a living Flexner-Jobling carcinoma cell with FHA-immune serum. *1*, 4½ minutes after beginning of incubation. No cytotoxic changes. *3*, 6½ minutes. Rounding up of cell. Nucleolus still faintly visible. *5*, 12 minutes. Beginning extrusion of cytoplasm in the form of a "blister" at lower left. Cytoplasmic particles by now are very large and appear to be coalescing. *7*, 17 minutes. Nucleolus no longer discernible. *11*, 54 minutes. Cytoplasm being rapidly extruded in the form of "blisters." Cell death has occurred by this time. *16*, 89 minutes. Normal cytoarchitecture has been completely lost.

DISCUSSION

The studies described demonstrate unequivocally the production of resistance to standard transplantable rat tumors in animals that have been immunized with FHA but that have had no prior exposure to tumors or tumor fractions of any kind. This tumor immunity has been shown by 3 kinds of experimental approaches.

FHA-immune animals have proved resistant to transplantation of standard, ordinarily easily transplantable, rat tumors. This resistance has been illustrated by 3 phenomena.

TABLE IX. NEUTRALIZATION FLEXNER-JOBLING CARCINOMA CELLS

	CELLS ADMINISTERED	SERUM	NO. OF RATS	TUMOR TAKES AND DEATHS
FHA-immune serum	560,000	FHA-immune	10	0
Normal serum	560,000	Control	9	7

Effect of incubation of Flexner-Jobling cells with FHA-immune and with normal serum. Transplantation, following incubation, was into normal animals.

1. Total immunity to development of tumors. Per cent immunity varies with the tumor tested, and probably also with the number and time sequence of inoculations with FHA and the time lapse from development of acute hemolytic crisis to transplantation of tumors. The resistance has varied from 25 per cent with the Walker 256 carcinosarcoma FHA-free to 90 per cent with the Miller hepatoma. Four of the tumor lines tested, the Walker 256 carcinosarcoma associated with FHA, the Walker tumor FHA-free, Miller hepatoma, and Novikoff hepatoma, have routinely resulted in 100 per cent tumor takes and deaths in control animals as well as in routine transplantation in normal stock animals.

2. Prolonged survival time. In experiments with the Novikoff hepatoma, those FHA-immune animals which did develop tumors resisted the lethal effects of the tumor more efficiently than the controls and survived 3 times as long after tumor transplantation.

3. Tumor modification. Altered growth pattern, gross appearance, and histocytologic morphology were observed in 4 of the 5 tumor takes in FHA-immune animals transplanted with the FHA-free Walker 256 carcinosarcoma. These changes, which have never been observed in control or normal animals with the same tumor, including encapsulation without infiltration, extensive necrosis, no ascites, tumor limited to 1 nodule, and a more differentiated appearance, are interpreted as manifestations of heightened host resistance. Similar modifications were seen in the Miller hepatoma and Flexner-Jobling carcinoma.

The second approach employed to demonstrate resistance to tumors in animals recovered from infection with FHA demonstrated that at least part of the mechanism of the tumor resistance is on a humoral basis. The effects of FHA-immune serum on the morphology of tumor cells in vitro were studied. The cytotoxic changes elicited by FHA-immune serum were definitive and clear. These changes are identical to those reported by others produced by heterologous antisera to normal and malignant cells. The specificity of the cytotoxicity was remarkable. Normal cells in the same incubatant mixture as the tumor cells were found to undergo none of the destructive changes of the tumor cells. Control sera were never observed to produce cytotoxic changes to either normal or tumor cells.

The third experimental approach, a neutralization test, provided additional evidence for the existence of humoral tumor immunity in FHA-immune animals. An experiment was performed that demonstrated the destruction of the viability of tumor cells incubated with FHA-immune serum and complement.

The 3 experimental approaches employed are included in the 6 listed by Snell[33] and Gorer[9] as accepted methods for demonstration of antitumor antibodies.

Two principles have been established by these studies, (1) the induction of immunity specifically against tumors in animals recovered from infection with a filterable, replicating agent, and (2) this induced immunity is not limited to 1 tumor but is generalized to a variety of tumors, in fact to every tumor that we have tested. (The tumors were obtained from 3 different sources.)

Excluding genetically disharmonious tumor-host systems, the induction of prophylactic and destructive immunity to tumors by antigenic material not derived from tumor cells has been demonstrated in only 2 isolated cases involving the induction of immunity to a specific tumor by the virus which induces that specific tumor. These studies, however, do not absolutely demonstrate that the immunity to the virus as such was responsible for the tumor immunity. In 1 case,[2] the source of the virus was obtained exclusively from ruptured tumor cells. In the other,[16] active immunization in the native host was not achieved but rather heterologous antisera were produced to virus preparations from normal and malignant mammary tissue. The possibility that the antiserum toxicity was directed to contaminant cellular components, either intimately or loosely associated with the virus, rather than exclusively to the virus, cannot be excluded. The production of immunity to more than 1 tumor by immunization with tumor cells or fractions from 1 tumor has been accomplished in a few cases. This immunity generally has been based on the genetic differential between genetically similar tumors and a genetically dissimilar host.

A possible objection to the interpretation and significance of the data presented stems from the admonishment of Hauschka[15] that inbred strains of animals be used that are genetically homogeneous and that spontaneous tumors of recent origin be employed as the antigenic material as well as the test system in tumor immunity studies. However, this caution is based on the fear that immunity induced by genetic differences between the host and tumor might be mistakenly attributed to specific tumor immunity. This frame of reference presupposes the induction of tumor immunity by tumor cells or fractions and therefore is not applicable to these studies. FHA induces immunity to tumors in animals which have had no exposure whatsoever with tumors of any kind, at any time.

Efforts to explain the occurrence of immunity to tumors in animals recovered from infection with FHA suggest 3 major hypotheses. The first assumes that the immunity is based on immunologic grounds, and that there are common antigen(s) shared by the tumor cells and the FHA agent—the common antigen being the FHA agent itself. If this were true, then all rat tumors, at least all the rat tumors tested, should contain the agent. The question immediately arises as to why the hemolytic anemia syndrome is not induced in animals transplanted with these tumors in other laboratories or with the tumors which we have termed free of the FHA agent in our laboratory. The existence of a masked form of FHA analogous to lysogenic phage, although possible, seems unlikely and is an unnecessary complicating postulate. An

alternative could be advanced that prior to inoculation into an animal that had recovered from FHA infection, the tumor was free of the agent, but that the FHA agent remained intracellularly in the tissues of the recovered animal. The FHA agent could then be assumed to enter or be absorbed onto the tumor cell transplanted into such an animal. (It has been demonstrated that tumor cells have a great affinity for the FHA agent.[29]) This animal, then, being immune to FHA and possessing antibodies to it, would destroy the tumor by an antigen antibody reaction directed against the agent which had entered or been absorbed by the tumor cells. This hypothesis is untenable, for immune cytotoxic reactions have been produced by FHA-immune serum incubated in vitro with FHA-free tumor cells that had not been passed in any animal exposed or inoculated with FHA.

A second hypothesis, although unlikely, is that the resistance to tumors induced by infection and recovery from FHA is not founded on immunologic grounds at all. It has been shown that the FHA agent itself is inactivated by serum from an animal recovered from the infection but not by normal serum. The presence of immune bodies in the serum of recovered animals is probably more than coincidental with the cytotoxicity of their serum for tumor cells. Furthermore, it is difficult to conceive of the nonimmunologic induction of a cytotoxin in the sera of animals recovered from FHA infection specific for tumor cells and without effect on normal cells.

The third hypothesis, and the one the authors favor, is that transplantable rat tumor cells contain antigen(s) in common with the FHA agent which are not present in normal tissues. This hypothesis is the simplest one in harmony with experimental data. Objections could be voiced that if there were an antigenic differential of considerable magnitude between tumors and normal tissues, the tumors, transplanted into normal animals, either would not grow or would regress after an initial period of growth. Numerous possible explanations could be offered to overrule this objection. It is possible that the cellular antigen provokes production of noncytotoxic antibodies or antibodies which cannot gain entrance into the effective reaction site necessary for cytotoxic action. It is also possible that the cellular antigen is incomplete or haptenic and thus could react with the formed antibody, but by itself could not induce the production of antibodies. A third possibility is that the cellular antigen is never released in undenatured form from the cell. It is quite obvious that such speculations are far removed from the level of knowledge concerning the mechanism of tumor immunity induced by FHA.

The possibility of the utilization of immunotherapy as either a primary modality or as an adjunct to surgical treatment has been overcast with pessimism in the past. If an immunologic foundation for cancer treatment be established, several contingencies must be met. First, there must be an antigenic differential between the tumor and the patient. Second, an antigen must be available capable of provoking production of antibodies lethal for tumor cells, but noninjurious to normal cells. Third, the antibodies must be capable of reaching the reaction site which proves lethal for the tumor cells (vascular,

extracellular, as well as intracellular barriers must be overcome). Under the conditions employed in these experiments, it would appear that FHA immunity meets these contingencies in the rat.

SUMMARY

The induction of immunity to tumors following recovery from infection with a replicating, filterable agent in the rat has been described. This induced immunity has been found effective against every tumor tested (6) and has been shown to be specific for tumors and noninjurious to normal cells. The immunity has been shown to be at least partially on a humoral basis.

Mechanisms of the induction and implications of this tumor immunity are discussed.

We gratefully acknowledge the excellent technical assistance of Mr. Anthony Mikalis and Mr. Bruce Watterson and the invaluable advice of Dr. Robert Marston on the performance of the in vitro cytotoxicity studies.

ADDENDUM

Since this paper was submitted an additional experiment with the fibrosarcoma has been performed with results demonstrating unequivocal resistance to the transplantation of this tumor in FHA-immune animals.

REFERENCES

1. Barrett, M. K., and Hansen, W. H.: Resistance to Tumor Implantation Induced by Red Cell Stromata, Cancer Res. **13**: 269, 1953.
2. Burmester, B. R.: The Cytotoxic Effect of Avian Lymphoid Tumor Antiserum, Cancer Res. **7**: 459, 1947.
3. Colter, J. S., Koprowski, H., Bird, H. H., and Pfeister, K.: Immunological Studies With Protein Fractions Isolated From Ehrlich Ascites Carcinoma Cells, Nature **177**: 994, 1956.
4. Colter, J. S., Kritchevsky, D., Bird, H. H., and McCandless, R. F. J.: In Vitro Studies With Antisera Against Tumor Cell Protein Fractions, Cancer Res. **17**: 272, 1957.
5. Dulaney, A. D., and Arnesen, K.: Cytotoxic Action of Antisera to Cell Components of Normal and Leukemic Mouse Spleens, Proc. Soc. Exper. Biol. & Med. **72**: 665, 1949.
6. Ellem, K. A. O.: Some Aspects of the Ascites Tumor Cell Response to a Heterologous Antiserum, Cancer Res. **18**: 1179, 1958.
7. Foley, E. J.: Antigenic Properties of Methylcholanthrene Induced Tumors in Mice of the Strain of Origin, Cancer Res. **13**: 835, 1953.
8. Goldstein, M. N.: The Cross Reactions of Antisera Prepared Against Four Human Cell Strains in Continuous Tissue Culture, J. Immunol. **79**: 113, 1957.
9. Gorer, P. A.: Some Recent Work on Tumor Immunity in Advances in Cancer Research, New York, 1956, Academic Press, Inc., vol. 4, p. 149.
10. Gorer, P. A., and Amos, D. B.: Passive Immunity in Mice Against C57BL. Leukosis E. L. 4 by Means of Iso-immune Serum, Cancer Res. **16**: 338, 1956.
11. Gross, L.: Immunological Relationship of Mammary Carcinomas Developing Spontaneously in Female Mice of a High Tumor Line, J. Immunol. **55**: 297, 1947.
12. Hanks, J. H., and Wallace, R. E.: Relation of Oxygen and Temperature in the Preservation of Tissues by Refrigeration, Proc. Soc. Exper. Biol. & Med. **71**: 196, 1949.
13. Hirsch, H. M., Bittner, J. J., Cole, H., and Iversen, I.: Can the Inbred Mouse be Immunized Against Its Own Tumor? Cancer Res. **18**: 344, 1958.
14. Horn, E. C.: Ascites Tumor Development. II. Cytotoxicity of Various Antisera Prepared Against Ehrlich Ascites Tumor Cell Components, Cancer Res. **16**: 595, 1956.
15. Hauschka, T. S.: Immunologic Aspects of Cancer: A Review, Cancer Res. **12**: 615, 1952.
16. Imagawa, D. T., Syverton, J. T., and Bittner, J. J.: The Cytotoxicity of Serum for Mouse Mammary Cancer Cells. I. The Effects of Admixture in Vitro Upon Homoiotransplantability, Cancer Res. **14**: 1, 1954.
17. Kalfayan, B., and Kidd, J. G.: Structural Changes Produced in Brown-Pearce Carcinoma Cells by Means of a Specific Antibody and Complement, J. Exper. Med. **97**: 145, 1953.

18. Kidd, J. G.: A Distinctive Substance Associated With the Brown-Pearce Rabbit Carcinoma. I. Presence and Specificity of the Substance as Determined by Serum Reactions, J. Exper. Med. **71**: 335, 1940.
19. Latta, H., and Kutsakis, A.: Cytotoxic Effects of Specific Antiserum and 17-Hydroxycorticosterone on Cells in Tissue Culture, Lab. Invest. **6**: 12, 1957.
20. Law, L. W., and Malmgren, R. A.: Studies on the "Cytotoxic" Property of Mammary Tumor Agent Antiserum, J. Nat. Cancer Inst. **11**: 1259, 1951.
21. Lewis, M. R., and Aptekman, P. M.: Antigenicity of Sarcomata Undergoing Atrophy in Rats, J. Immunol. **67**: 193, 1951.
22. Lumsden, T.: On Cytotoxins Lethal to Nucleated Mammalian Cells Normal and Malignant, Am. J. Cancer **31**: 430, 1937.
23. Lumsden, T.: Tumor Immunity: The Effects of the eu- and Pseudoglobulin Fractions of Anti-cancer Sera on Tissue Cultures, J. Path. & Bact. **34**: 349, 1931.
24. Martinez, C., Aust, J. B., Bittner, J. J., and Good, R. A.: Continuous Growth of Isotransplants of a Mammary Tumor Associated With the Development of Immunity in Mice, Cancer Res. **17**: 205, 1957.
25. Miller, D. G., and Hsu, T. C.: The Action of Cytotoxic Antisera on the HeLa Strain of Human Carcinoma, Cancer Res. **16**: 306, 1956.
26. Nungester, W. J., and Fisher, Helen: The Inactivation In Vivo of Mouse Lymphosarcoma 6C3HED by Antibodies Produced in a Foreign Host Species, Cancer Res. **14**: 284, 1954.
27. Prehn, R. T., and Main, J. M.: Immunity to Methylcholanthrene Induced Sarcomas, J. Nat. Cancer Inst. **18**: 769, 1957.
28. Prince, J. E., Fardon, J. C., Nutini, L. G., and Sperti, G. S.: Induced Resistance to an Indigenous Transplantable Mouse Tumor, Cancer Res. **17**: 312, 1957.
29. Sacks, J. H., and Egdahl, R. H.: A Multipotent Filterable Agent in Rats Capable of Producing Acute Hemolytic Anemia, Delayed Tumor Development and Resistance to Standard Transplantable Tumors, Clin. Res. **8**: 61, 1960.
30. Sacks, J. H., and Egdahl, R. H.: Protective Effects of Immunity and Immune Serum on the Development of Hemolytic Anemia and Cancer in Rats, S. Forum 10: 22, 1960.
31. Schrek, R., and Preston, F. W.: Toxicity of Homologous Immune Serum to a Transplantable Tumor: Studies Using Phase Microscopy and Cinemicrography, J. Nat. Cancer Inst. **16**: 1021, 1956.
32. Schrek, R., and Preston, F. W.: Cytotoxicity of a Homologous Immune Serum to a Transplantable Rat Tumor by the Method of Unstained Cell Counts, Cancer Res. **17**: 102, 1951.
33. Snell, G. D.: Incompatibility Reactions to Tumor Homotransplants With Particular Reference to the Role of the Tumor—A Review, Cancer Res. **17**: 2, 1957.
34. Wissler, R. W., and Flax, M. H.: Cytotoxic Effects of Antitumor Serum, Ann. New York Acad. Sc. **69**: 773, 1957.

(For discussion, see page 270.)

INTRACYTOPLASMIC INCLUSIONS IN COLON CARCINOMA

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THE microscopic anatomy of human carcinoma is generally characterized by the morphology of cells and nuclei, irregularity of organ pattern, invasiveness, lack of polarity, and other features which have to do with the cell as a unit and its relationship to a group of similar cells. Recent investigations have begun to define intracellular properties in the hope that carcinomatous differences may be identified. Enzyme systems have generally been found to demonstrate some quantitative differences. In the study of colon carcinoma, Wattenberg[16] used histochemical techniques to investigate enzyme content and demonstrated differences between colon cancer and normal colon epithelium. Leuchtenberger[10, 11] studied polypoid tumors and cancer of the colon and consistently found inclusions in the cytoplasm which stained for desoxyribose nucleic acid (DNA) with the Feulgen reaction. Helwig[8] found similar inclusions in the epithelium of chronic ulcerative colitis. Cole and Holden[2] studied 2 patients who underwent spontaneous regression of colon polyps. They also reported the finding of Feulgen-positive bodies in the cytoplasm.

The study of the intracytoplasmic morphology of tumors has largely been confined to mouse, frog, rabbit, and other experimental tumors. Bernhard, Guerin, and Oberling[1] and Dmochowski[5] studied the cells of mouse mammary cancer with the electron microscope and demonstrated virus-like inclusion bodies in the cytoplasm. These bodies were spheres of 500 to 1,500 Å with an internal dense region of 150-330 Å, and an outer pale zone with a well-defined limiting membrane. Human leukemia has been studied by Dmochowski[6] who reports the finding of small objects, morphologically resembling viruses, in the cytoplasm of leukemic lymph nodes from human beings. By means of the electron microscope, we have studied the fine structure of colon cancer in its primary or secondary location in 13 patients. This report demonstrates 3 types of unusual intracytoplasmic inclusions.

MATERIALS AND METHODS

Tissue for this study was obtained from proctoscopic biopsy and from biopsy at the time of operative resection of primary colon cancer and its hepatic, peritoneal, and subcutaneous metastases. The tissues were immediately immersed in Dalton's[3] chrome-osmic fixative for 30 minutes, after which dehydration and methacrylate embedding was carried out. Sections were cut at 200 to 300 Å thickness with a Porter-Blum microtome utilizing a glass knife, and examined in an RCA-EMU-3D electron microscope. Colon epithelium from patients without carcinoma ("normal") and from uninvolved colon in patients with colon carcinoma ("noncancer") was also examined in a similar fashion.

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Fig. 1.

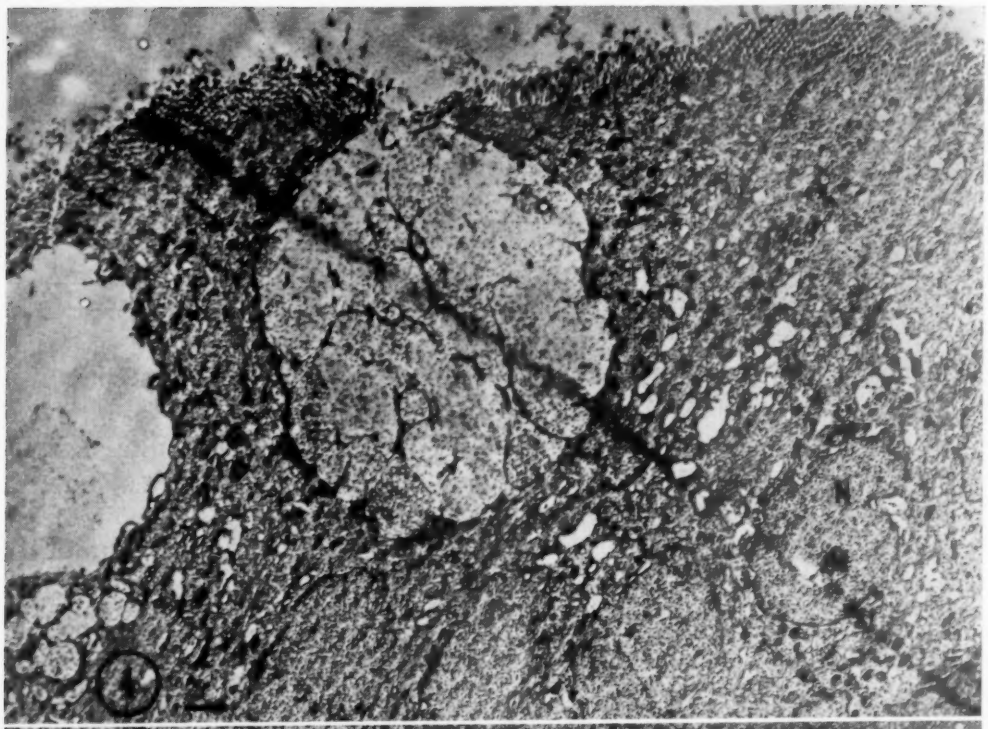

Fig. 2.

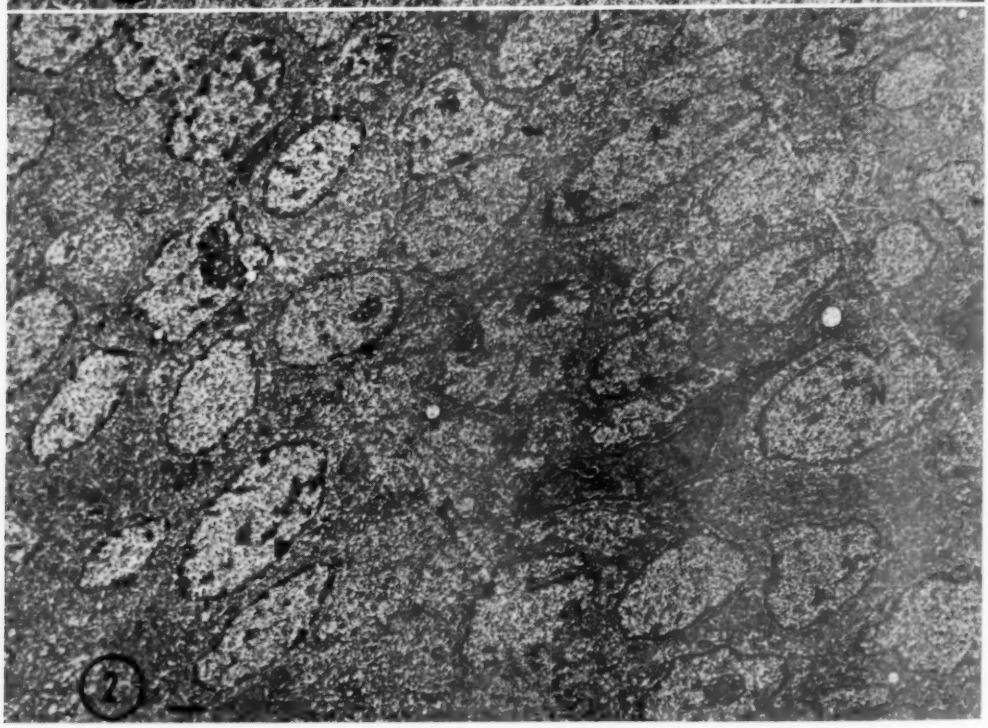

Fig. 1.—Noncancer transverse colon mucosa. (×5,000.)
Fig. 2.—Carcinoma of the rectum. (×4,500.)

RESULTS

In the accompanying 8 illustrations* are presented data representative of our findings in this study.

Fig. 1 demonstrates a low-power view of "noncancer" colon mucosa. There are numerous microvilli on the lumen side of the cell. The nuclei are roughly oval in shape and of approximately equal size. There is contour irregularity and frequently 1 nucleolus may be seen. Within the cytoplasm of 1 cell is a large droplet of mucin, apparently about to discharge into the lumen of the crypt. Numerous vacuoles, mucin collections, and mitochondria may be seen in the cytoplasm. Intercellular membranes may be seen dividing the cells. No distinguishable difference between "noncancer" and "normal" epithelium has as yet been observed.

Fig. 2 demonstrates a low-power view of a sheet of carcinoma cells removed from the rectum. The nuclei are irregular. Chromatin material is irregularly clumped and the nuclei vary in density. Intercellular membranes are distinguishable. The cytoplasm is of variable density. Mitochondria are numerous and intracytoplasmic vacuoles are small and relatively infrequent. No mucin is present in this illustration.

Fig. 3 demonstrates a metastasis to the peritoneum. Here the cells are growing in small sheets lying in large fluid (?) spaces. One of the cells demonstrates a tendency to form microvilli. The nuclei are of variable density with clumped chromatin and irregular shape. There are mitochondria, small vacuoles, and no mucin.

Fig. 4 demonstrates 2 cells growing in dense fibrous tissue taken from a wound implant to the abdominal wall. Here the cells appear to be growing in small fluid (?) spaces. The nuclei are smooth and fairly homogenous. The cytoplasm is dense, vacuoles are few, mitochondria are present, and mucin is absent.

Fig. 5 is a view of nucleus and cytoplasm from primary carcinoma of the rectum. The white line crossing the plate diagonally is an artifact produced by irregularity in the knife edge. The nucleus shown here is pale, homogenous, and irregular. The cytoplasm is moderately dense with several double membranes which suggest the membranes of ergastoplasm or mitochondria. Vacuoles are rare. Scattered throughout the cytoplasm in regular fashion are several dense bodies varying in size from 0.25 to 1.0 μ. These bodies are surrounded by an empty space and have several tiny projections in a radial fashion which extend from the body to the periphery. We have called these inclusions "degeneration bodies" or "D bodies."

Fig. 6 demonstrates a D body at higher magnification. An irregular nucleus lies adjacent to this D body. The nuclear contour is irregular and the double nature of its limiting membrane is visible. We have not seen a well-defined membrane limiting the periphery of the D body vacuole. There is no well-defined internal structure to the D body.

*In the accompanying illustrations, each horizontal bar represents one micron. N = nucleus, M = mitochondrion, D = degeneration body, V = virus-like particle, P = provirus-like particle.

Fig. 3.

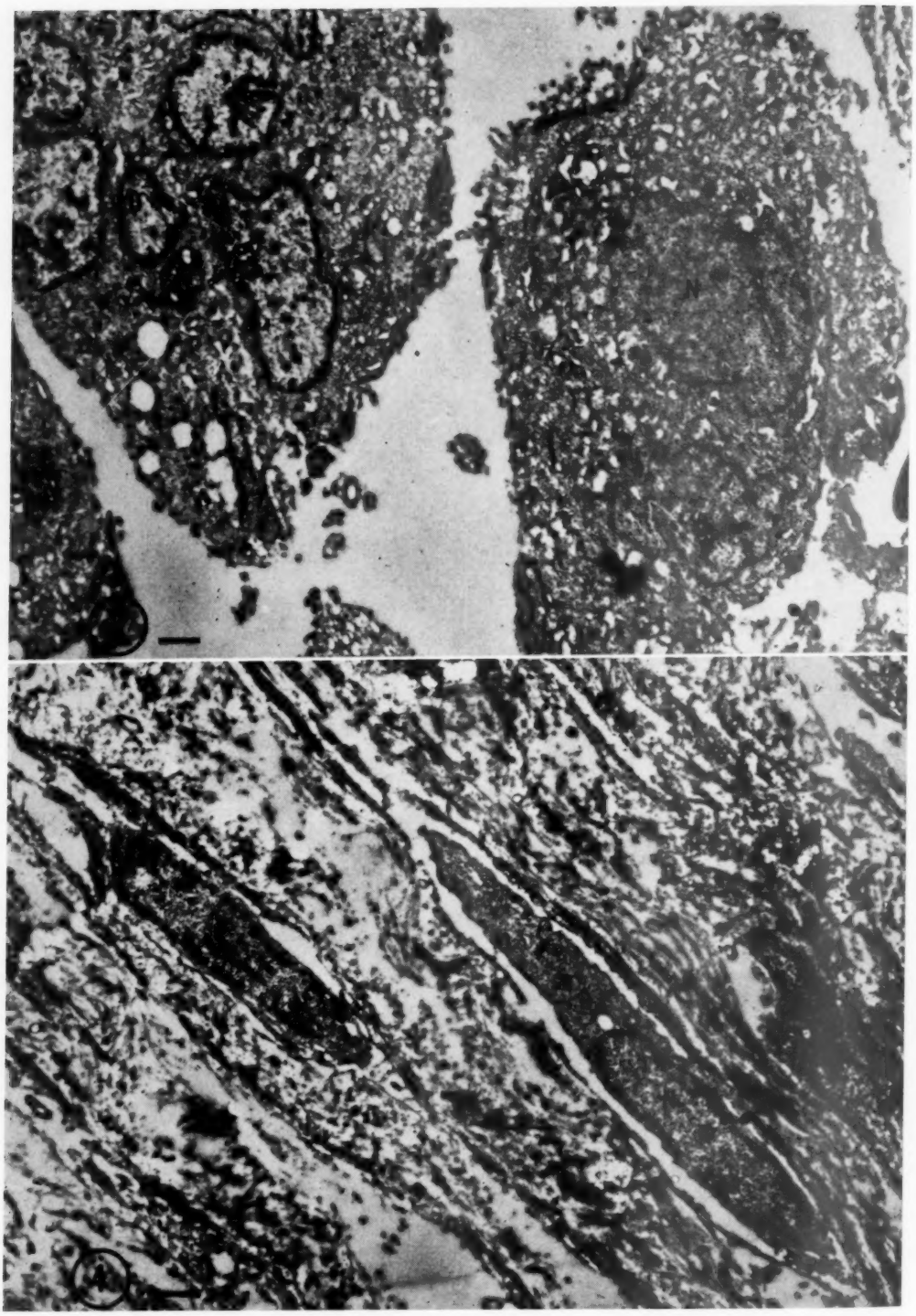

Fig. 4.

Fig. 3.—Peritoneal metastasis from carcinoma of the sigmoid colon. (×5,500.)
Fig. 4.—Wound implant metastasis from carcinoma of the sigmoid colon. (×7,000.)

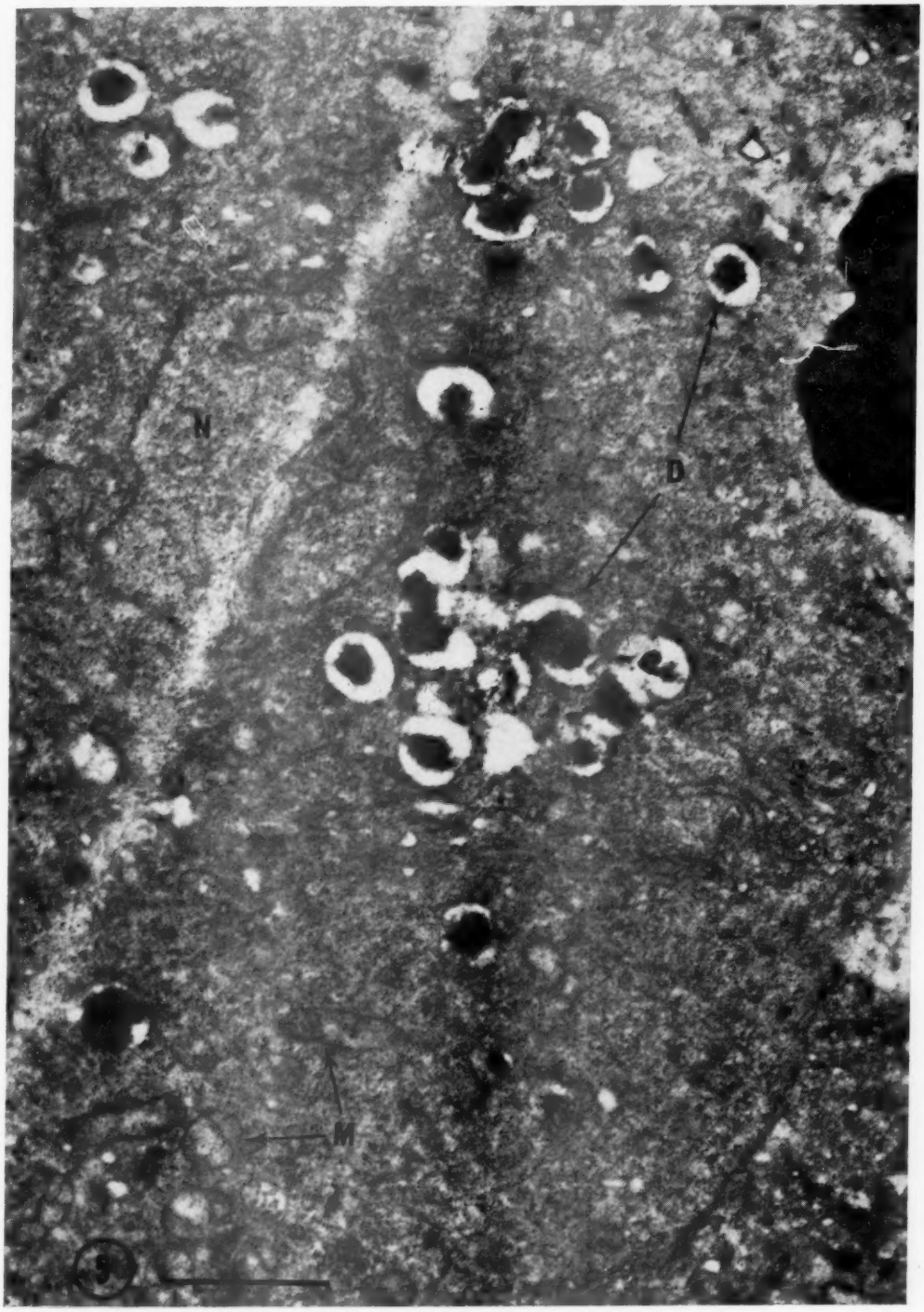

Fig. 5.—Adenocarcinoma of the rectum. (×22,500.)

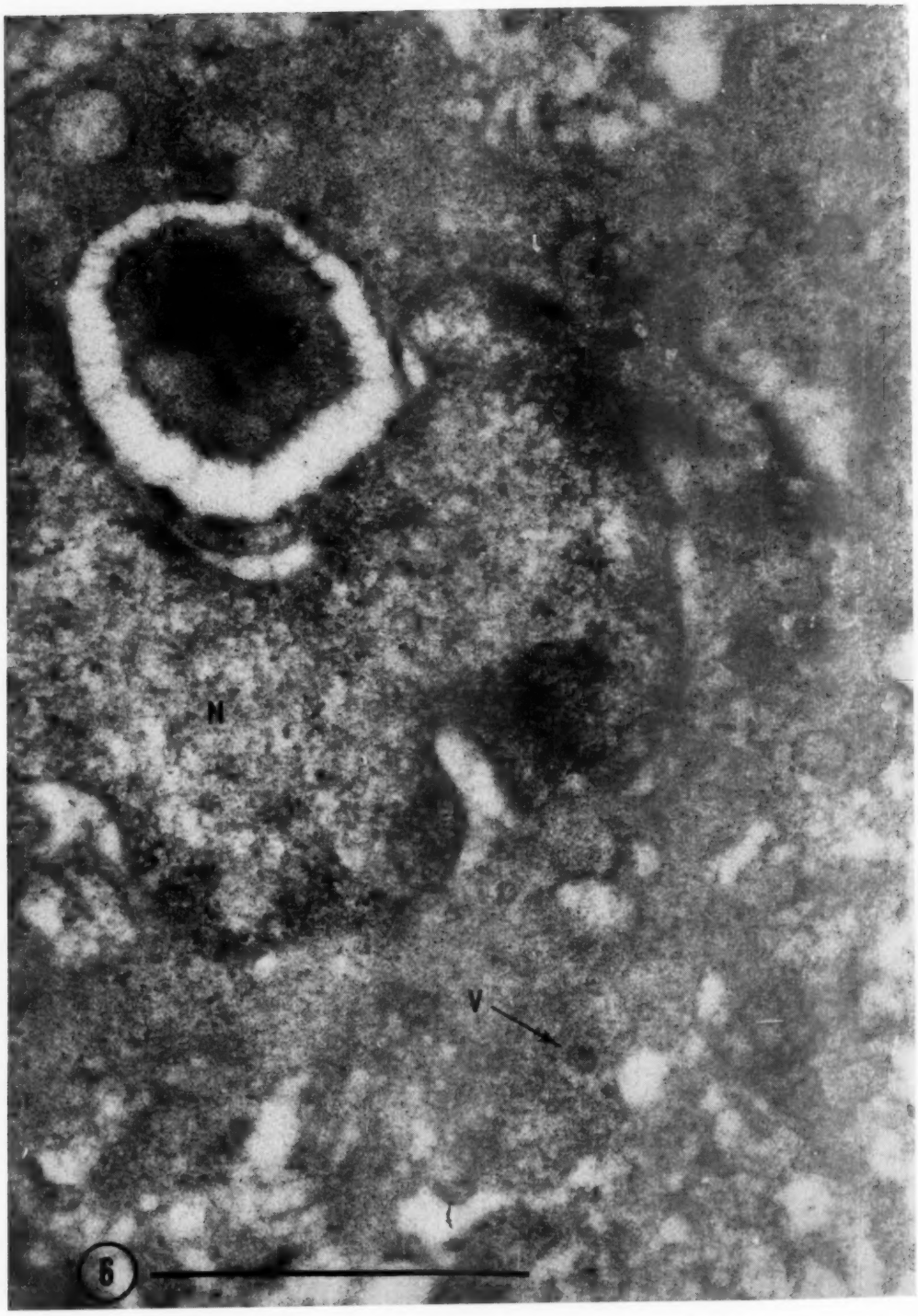

Fig. 6.—Adrenocarcinoma of transverse colon. (×50,000.)

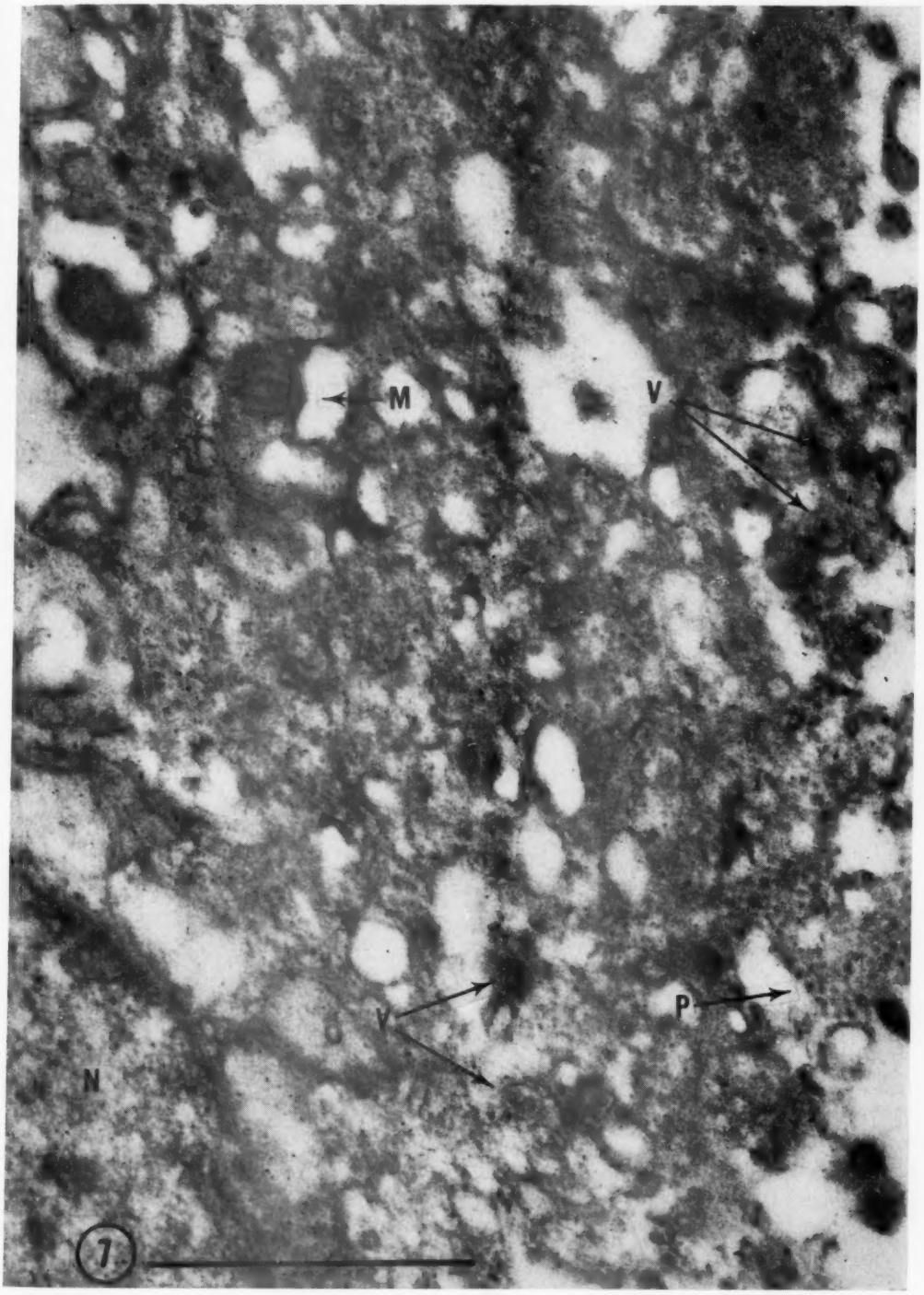

Fig. 7.—Adenocarcinoma of transverse colon. (×43,500.)

Fig. 7 demonstrates the cytoplasm of a primary carcinoma of the transverse colon. A portion of the nucleus may be seen at *N*. At *M* a vacuolated mitochondrion is seen with its cristae. Several vacuoles are seen. Throughout the

Fig. 8.—Adenocarcinoma of sigmoid colon. (×44,500.)

cytoplasm are numerous, small, ovoid bodies which have a peripheral membrane, a dark central core, and a lighter area between the central core and the peripheral membrane. These bodies range in size from 1,200 Å in their long axis to 900 Å in the transverse axis. Several such particles are shown by the arrows. These particles have structure similar to those in photographs published by other workers and called virus-like particles. We have thus elected to call these inclusions "virus-like particles" or "V particles." At *P* there is a cluster of small, round, electron-dense particles of rather uniform shape which we have arbitrarily called "P particles." These particles are of the approximate size of the central core of the V particle and are considerably larger than the small aggregations of RNA material characteristic of ergastoplasmic granules.

Fig. 8 demonstrates pale cytoplasm from a recurrence of primary carcinoma of the sigmoid growing in the colon wall. In this illustration there is a degenerate mitochondrion at *M* with well-defined cristae. Scattered throughout the illustration are aggregates of ovoid particles, some of which have a dense central core and fulfill the criteria for being V particles.

DISCUSSION

Cole and Holden[2] and Leuchtenberger[10, 11] have demonstrated the presence of intracytoplasmic inclusions in the epithelium of colon polyps and colon cancer. We, too, have observed such inclusions stainable by the Feulgen technique in paraffin section. The only bodies of comparable size which we have observed by electron microscopy are the D bodies. Leuchtenberger and colleagues[11] have presented 1 electron microscope picture which demonstrates bodies similar to our D bodies. They concluded that the Feulgen-positive bodies seen with the light microscope are identical to those presented in the electron micrograph and that these inclusions are probably of viral origin. Structures similar to the D body have been observed in placenta,[4] liver,[15] kidney,[14] human skin in tissue culture,[12] and human cancer in tissue culture.[9] The appearance of these objects suggests that they are degenerate bits of cytoplasm without internal structure. Their density indicates that they are of a substance which has an affinity for osmic acid, and thus are very likely to be fragments of nuclear material (DNA) or a lipid droplet of unknown composition. The appearance of the clear space surrounding the dense central area suggests that at some time, probably during fixation, shrinkage of the cytoplasmic material has occurred. We feel that this particle is probably unrelated to the presence of viruses.

We have not observed V particles in "normal" or "noncancer" epithelium as yet. The V particles demonstrated here are morphologically similar to electron micrographs of viruses found in such diverse diseases as animal tumors and animal and human infections.[5-7, 13] It is, of course, unwarranted at the present time to assume that causal or etiological relationships exist between the V particle and malignancy, though it is of interest that we have found such particles in all cancers examined.

CONCLUSIONS

Electron microscopy of carcinoma of the colon at its primary and secondary sites, has demonstrated 3 unusual intracytoplasmic inclusions:

1. A large, dense body with peripheral space—the D body which we think represents an aggregate of degenerate cytoplasm or nucleoplasm.

2. A small, ovoid body with outer membrane and dense central core. This has the morphologic characteristics of a virus and has been called a "V particle."

3. Aggregates of small, dense granules of the size of the central core of the V particle which we have called "P particle."

We are indebted to Mrs. Ellen Miles for technical assistance in the preparation of these tissues.

REFERENCES

1. Bernhard, W., Guerin, M., and Oberling, Ch.: Mise en évidence de corpuscles d'aspect virusal dans différentes souches de cancers mammaires de la souris, Acta Unio internat. contra cancrum **12**: 544, 1956.
2. Cole, Jack W., and Holden, William M.: Post-colectomy Regression of Adenomatous Polyps of the Rectum, A. M. A. Arch. Surg. **41**: 385, 1959.
3. Dalton, A. J.: A Chrome-Osmium Fixative for Electron Microscopy, Anat. Rec. **121**: 281, 1955.
4. Dempsey, E. W., and Wislocki, G. B.: Electron Microscopic Observations on the Placenta of the Cat, J. Biophys. & Biochem. Cytol. **2**: 743, 1956.
5. Dmochowski, L.: A Biological and Biophysical Approach to the Study of the Development of Mammary Cancer in Mice, Acta Unio internat. contra cancrum **12**: 581, 1956.
6. Dmochowski, L., Grey, C. G., Sykes, J. A., Schullenberger, C. C., and Howe, C. D.: Studies on Human Leukemia, Proc. Soc. Exper. Biol. & Med. **101**: 686, 1959.
7. Fawcett, D. W.: Electron Microscopic Observations on Intracellular Virus-like Particles Associated with the Cells of the Lucké Renal Adenocarcinoma, J. Biophys. & Biochem. Cytol. **2**: 725, 1956.
8. Helwig, F. C.: Epithelial Cytoplasmic Inclusion Bodies of Possible Viral Origin in Clinically Early Ulcerative Colitis, Dis. Colon Rectum **2**: 23, 1959.
9. Ivler, D.: Unpublished data.
10. Leuchtenberger, C.: Cytoplasmic "Inclusion Bodies" Containing Desoxyribose Nucleic Acid (DNA) in Cells of Human Rectal Polyps, Lab. Invest. **3**: 132, 1954.
11. Leuchtenberger, C., Leuchtenberger, R., and Lieb, E.: Studies of the Cytoplasmic Inclusions Containing Desoxyribose Nucleic Acid (DNA) in Human Rectal Polypoid Tumors Including the Familial Hereditary Type, Acta genet. & Statistica Medica **6**: 291, 1956.
12. Menefee, M.: Unpublished data.
13. Morgan, C., Rose, H. M., and Moore, D. H.: Structure and Development of Viruses Observed in the Electron Microscope, J. Exper. Med. **104**: 171, 1956.
14. Mueller, C. B.: Unpublished data.
15. Simpson, W.: Personal communication.
16. Wattenberg, L. W.: A Histochemical Study of Five Oxidative Enzymes in Carcinoma of the Large Intestine in Man, Am. J. Path. **35**: 113, 1959.

Discussion

(Papers by Sacks, Clark, and Egdahl; Mueller, Menefee, and Ivler)

DR. JACK W. COLE (Cleveland, Ohio).—I would like to address my comments to Dr. Mueller and congratulate him on what I think is a very beautiful piece of work and to express publicly my appreciation for the opportunity to read his abstract and paper prior to presentation this morning. To my way of thinking, this work adds to the increasing amount of evidence, albeit circumstantial, that carcinomas of the colon and rectum may well be caused by an infectious virus. There seems to be little doubt also that the small particles, the "V-like particles" that Dr. Mueller has shown, are in keeping with the general morphology of known viruses. I would question the term "degeneration body" for the larger particles that Dr. Leuchtenberger described in 1954. The reason I say this is that we have not as yet refuted the fact that these are DNA-positive particles and, as Dr. Mueller mentioned in his opening remarks, DNA is not a normal constituent of cytoplasm. Degenerative products of a normal cytoplasmic constituents would not be expected to be DNA-positive. The other point that I think may be of some significance

is that these so-called D bodies, or Leuchtenberger inclusion bodies, have almost invariably been situated in the infranuclear position and their relatively constant size, shape, and position would speak against their being products of nuclear degeneration. The possibility that they may be a result of a virus infection rather than the actual virus itself cannot be excluded. Virus infections can produce rather marked changes in the cell, not only in the cytoplasm, but also in the nucleus, and there is reason to believe that the increased DNA content of the nucleus in some virus-induced tumors may be a result of this virus infestation.

DR. GEORGE E. MOORE (Buffalo, N. Y.).—I think we surgeons will have to learn a great deal more about virology in the next several years. Dr. Sacks' paper, in my opinion, is one of the outstanding papers of this meeting. The field of tumor viruses is very exciting and I hope more young surgeons will interest themselves in it since inevitably the finding of genetic material which induces cancer will affect cancer immunology, chemotherapy, and prevention. Dr. Sacks has attacked several aspects of this problem which an experienced virologist would not.

I would like to discuss briefly another animal tumor-virus system. This concerns the polyoma virus which was originally isolated from leukemic mice by Dr. L. Gross. It is an amazing tumor virus in that it will provoke the development of different kinds of tumors in a single animal and it will cross species barriers. The virus can be replicated, millions-fold, in tissue culture and therefore the virus can be isolated and better studied. A vaccine to the polyoma virus has been prepared which gives excellent protection. One animal developed 9 different kinds of carcinomas and sarcomas. The speed with which these tumors can be induced is remarkable. A substrain of this virus will provoke huge tumors of the kidney in just 10 days after injection into newborn animals. Inclusion bodies occur in cells injected with the polyoma virus.

Dr. James Grace, of our institute, has induced tumors in over 100 mice with cell-free extracts of human malignancy. The importance of this work is self-evident.

DR. DAVID M. HUME (Richmond, Va.).—I just wonder if Dr. Sacks would tell us whether the FHA agent would be effective against a tumor which had already developed in the animal. He has told us he can immunize animals against the future development of tumors, but I wonder if he can do anything to a tumor which is already growing in the animal.

DR. SACKS (closing).—I should like to consider first Dr. Moore's comment concerning immunity to the polyoma virus. Immunity to polyoma virus is immunity to the virus per se. Immunity to the virus presumably inactivates the virus and therefore prevents the subsequent formation of tumors. The system which I described involves the destruction of tumor cells and, therefore, the 2 are not comparable.

In answer to Dr. Hume's question—we have some preliminary studies on established growing tumors, but the results are not yet complete and it would be difficult to answer with certainty. However, it does appear that the agent (or immunity to the agent) will destroy a certain per cent of growing established tumors in the rat.

Rats ordinarily develop a low per cent of spontaneous malignant tumors. A very large colony of rats would be required to compare the incidence of spontaneous neoplasms in FHA-immune and control rats. One experimental approach to approximate the incidence of spontaneous malignant neoplasms in FHA-immune and control animals is now in progress. This study consists of a comparison of the incidence of tumors arising in FHA-immune and control rats treated with a potent carcinogen (methylcholanthrene).

DR. MUELLER (closing).—I want to thank you for the privilege of presenting this work. This is, of course, only introductory. The discussors have been very kind and we are in the process of attempting to culture some of these cells and to find whether or not any of these agents are present in tissue culture and are transmissible from one culture to another. At the present time, we have been unable to grow normal colon epithelium but have had some success in growing carcinoma.

STUDY ON TRANSPLANTATION IMMUNITY AFTER TOTAL BODY IRRADIATION: CLINICAL AND EXPERIMENTAL INVESTIGATION

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(From the Departments of Surgery, Medicine, Pathology, Radiology and Immunology, The Peter Bent Brigham Hospital and Harvard Medical School)

IONIZING radiation has been used to depress the immune response and antibody production for many years. The delay in the antibody response to bacterial antigens following irradiation was described by Hektoen in 1915.[5] Krebs and associates described its effect in the transmission of leukemia to irradiated hosts in 1930.[7] Like other reticuloendothelial depressant agents, such as trypan blue, thorium dioxide, nitrogen mustard, and cortisone, irradiation of the host tends to prolong the survival of homografts.[2] Moreover, the toxicity of an effective dose of total body irradiation, manifest chiefly on the host hematopoietic system, was found to be preventable by lead shielding of a small area such as spleen or a long bone.[6] In lieu of lead shielding, a further refinement is total replacement of the reticuloendothelial and hematopoietic tissues from isologous, homologous, or heterologous donors after the required heavy dose of total body irradiation.[8] The observation that skin grafts from the hematopoietic tissue donor survived permanently completed the experimental design which has been the basis for a variety of experiments.[9] Although certain cells and tissues, for example lymphocytes, splenic tissue, and marrow, can be shown to proliferate and react against a homologous irradiated host, there is no evidence that a transplanted kidney is capable of behaving in this way.[15] Experiences with humans and rabbits subjected to total body irradiation and marrow infusion followed by homografting form the basis of this report.

HUMAN EXPERIENCE

To date, 6 patients have been subjected to varying doses of total body irradiation in an effort to alter the immune state preparatory to kidney homotransplantation (Table I).

CASE 1.—G. L. was a 35-year-old mother of 2, blood type AB Rh positive, who underwent removal of a ruptured solitary pelvic kidney. On the nineteenth day, following 3 hemodialyses, 600 r total body irradiation, calculated in air to the midplane of the body, was delivered with a 2.0 kv. unit over a 3⅓-hour period.* The patient was transferred to a sterile operating room, and all attending personnel were put on strict surgical precautions. In the following 48 hours, 36 billion marrow cells were infused from 11 donors,

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Read at the Twenty-first Annual Meeting of the Society of University Surgeons, Minneapolis, Minn., Feb. 11-13, 1960.

*Irradiation carried out by Dr. Joseph H. Marks, New England Deaconess Hospital.

TABLE I

PATIENT	DATE	DIAGNOSIS	PROCEDURE	OUTCOME
G. L., 31, female	March, 1958	Absent kidneys (rupture solitary kidney)	600 r, 2.0 mev. Pooled marrow from siblings, unrelated adults, and kidney donor. Kidney donor was unrelated infant	Lived 32 days after x-ray, 28 days after transplant Renal function present Death was hematologic
N. W., 12, male	July, 1958	Absent kidneys (rupture solitary kidney)	700 r, 2.0 mev. Bone marrow from mother (skin from mother). Fetal spleen and liver cells	Lived 25 days after x-ray Skin intact Death hematologic
J. R., 24, male	Jan., 1959	Chronic glomerulonephritis	450 r, 250 kv. Split dosage 7-day interval. No marrow. Diovular twin kidney donor	Living and well 12 months Skin graft rejected at 7 months
R. D., 56, male	June, 1959	Acute and subacute glomerulonephritis	450 r, 250 kv. Split dosage 7-day interval. No marrow. Kidney donor unrelated infant	Lived 19 days after x-ray, 11 days after transplant. No urine output Died bacterial and uremic death
M. C., 15, female	Aug., 1959	Subacute and chronic glomerulonephritis	400 r, 250 kv. Split dosage 7-day interval. No marrow. Kidney donor father	Lived 22 days after x-ray, 14 days after transplant. Good volume output Lowering of BUN Died bacterial death
G. C., 18, female	Sept., 1959	Chronic glomerulonephritis	250 r, 250 kv. Single dose. No marrow. No homograft	Died 18 days after x-ray of uremia and infection

representing all major blood types. On the third postirradiation day, a renal homograft from an A positive, 4-year-old female* was anastomosed to the femoral vessels in the right groin, with a skin ureterostomy. The kidney donor contributed 170 million cells. The transplanted kidney secreted 500 c.c. for the first 48 hours. An 8-day anuric period followed, during which renal biopsy revealed normal vascularization. The leukocyte count fell to 500 per cubic millimeter, the platelets to 28,000 per cubic millimeter, and hematocrit to 23 per cent. In spite of hemodialysis and fresh platelet transfusions from multiple donors, mucosal and nasal bleeding occurred. Seventy billion more marrow cells from a cadaver and 5 live donors (total 106 billion marrow cells) were given 1 week postoperatively. On the eleventh day, urine secretion returned and reached a peak of 1,750 c.c. on the sixteenth postoperative day, being over 1,000 c.c. on 4 consecutive days. At this high point in her course, the patient was taking oral fluids, was free from discomfort, and the wounds were healing well. Electrolyte pattern was normal and all cultures from the urine and wounds continued to be negative. However, epilation became apparent, and the hematologic state never improved. Petechiae occurred with mucosal bleeding in spite of continued fresh platelet transfusions. Stomatitis and parotitis were controlled by hygienic measures. Severe generalized, uncontrollable convulsions occurring on the seventeenth postoperative day lasted for 48 hours. Spinal tap was negative. The postconvulsive course was steadily downward. Bleeding into the renal pelvis blocked the ureter and required open drainage.

*Nephrectomy performed by Dr. Donald D. Matson, Children's Hospital, in the establishment of a ventricular-ureteral shunt.

Urine secretion decreased. Bradycardia and hypotension led to death, 32 days after total body irradiation and 28 days after transplantation. Autopsy revealed sufficient numbers of leukocytes with the female chromatin marker to indicate recovery of the patient's own marrow and no evidence for marrow graft survival. The kidney homograft showed no evidence of rejection. Pulmonary edema, foci of bronchopneumonia with necrosis of the alveolar walls, and hemorrhage were the apparent causes of death. The clinical course of this patient is charted in Figs. 1-3.

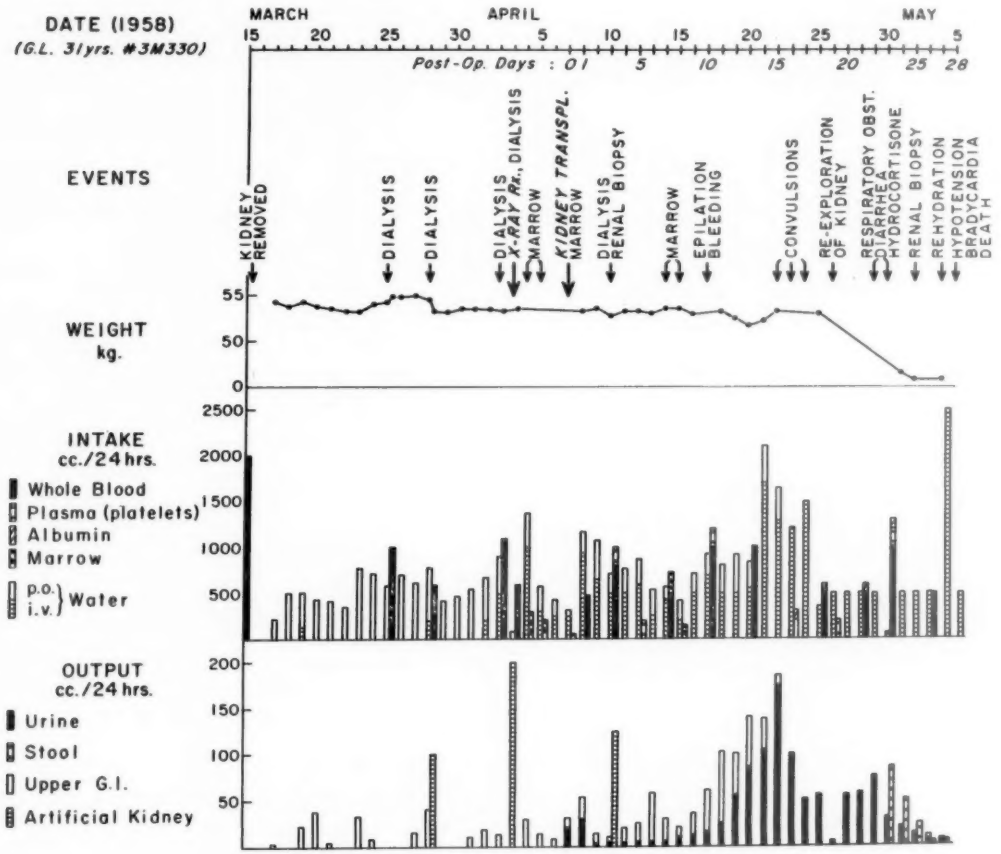

Fig. 1.—Clinical course of patient in Case 1.

CASE 2.—N. W. was a 12-year-old boy whose solitary kidney was ruptured by blunt trauma. Because there had been no marrow graft survival in Case 1, a higher dose of x-ray (700 r) was delivered (by Dr. Joseph H. Marks) from the same 2 million volt source. On the following day, the boy received 7.4 billion marrow cells and 7 days later 3.1 billion cells from his mother, the prospective kidney donor. An additional 1.5 billion fetal liver and spleen cells were infused 6 days later. Since there was no evidence for marrow graft survival and there was persistent bleeding, it was felt inadvisable to sacrifice one of the mother's kidneys. The patient died of bilateral bronchopneumonia and intestinal hemorrhage 25 days after x-ray. The lymphoid tissue and bone marrow were hypoplastic. A maternal skin graft which had been in place for 20 days was still intact.

CASE 3.—J. R., 26-year-old male, one of fraternal twins, was suffering from terminal glomerulonephritis. He shared 26 blood group antigens with his brother and had accepted a fraternal skin graft for 40 days. First- and second-set skin grafts from patient to healthy twin were rejected at 24 days and 12 days, respectively. Following 250 r and

200 r total body irradiation at 7-day intervals from a 250 KVP constant potential source, a renal transplant was performed retroperitoneally in the right lower quadrant to the iliac vessels with a ureterocystostomy, according to a technique previously described.[11, 12] The graft functioned immediately. The leukocyte count fell to less than 50 per cubic millimeter, the platelets to 58,000 per cubic millimeter and the hematocrit to 18 per cent. Massive perirenal infection developed, requiring emergency bilateral nephrectomy on the eleventh postoperative day.* The blood elements returned to normal levels by 21 days, after which the patient made a complete recovery. Although the skin homograft from his brother was rejected at 7 months over a 6-week period with a chronic type of rejection pattern, the renal graft continues to function well. The clinical course is charted in Fig. 4.

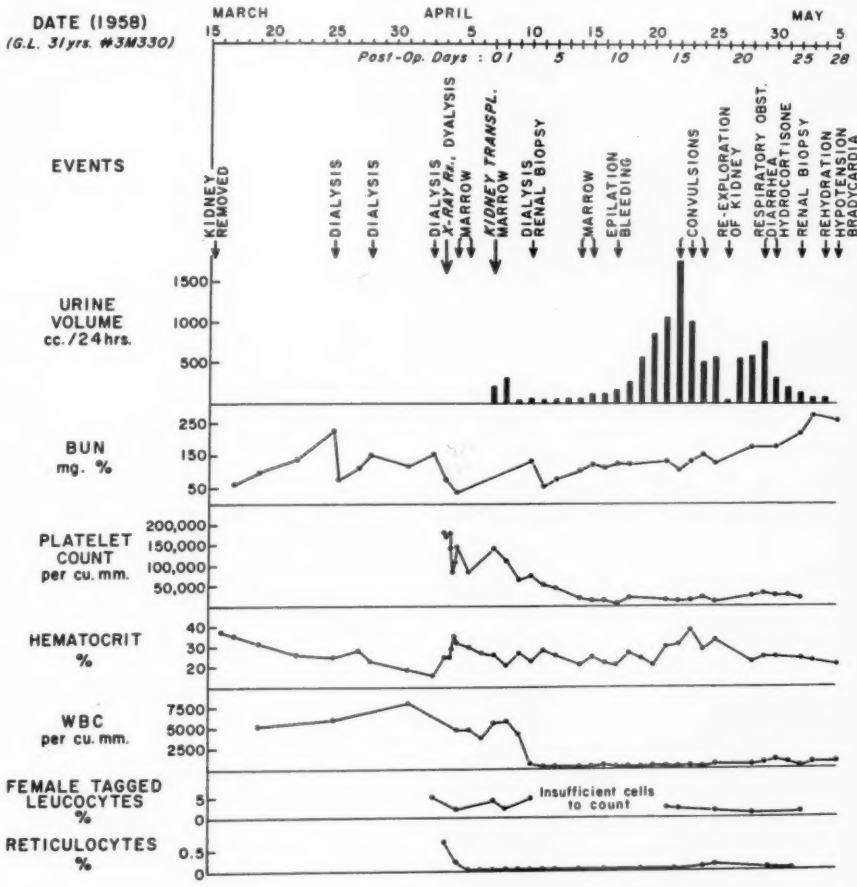

Fig. 2.—Clinical course of patient in Case 1.

CASE 4.—R. D., a 55-year-old physician, O positive blood type, with acute and subacute glomerulonephritis, was subjected to 250 r and to 200 r at 7-day intervals from a 250 KVP constant potential source. A donor kidney from a 6-year-old female,† B positive blood type, was placed in the right lower quadrant retroperitoneally and anastomosed to the iliac vessels. A ureteroureteral anastomosis was performed. The hematologic response was severe, the leukocyte count falling to 100 per cubic millimeter, the hematocrit to 25 per cent, and the platelets to less than 50,000. The renal homograft never functioned,

*Operation performed by Dr. J. Hartwell Harrison.

†Nephrectomy performed by Dr. Donald D. Matson in the course of a ureteroventricular shunt.

and the patient died of renal insufficiency 11 days after operation. At postmortem a severe cortical necrosis was evident, probably a result of the blood group incompatibility. The vascular and ureteral anastomoses were patent.

CASE 5.—M. C. was a 15-year-old girl with subacute and chronic glomerulonephritis who received 250 r initially and 7 days later 150 r, a total of 400 r in 2 doses at a 7-day interval. A kidney homograft from her father who shared the major blood group antigens was transplanted to the right lower quadrant with the same technique as employed in Case 3.* In spite of good urine output for 12 days, the patient died on the fourteenth day from bronchopneumonia. Her hematologic response to x-ray was severe with a white count less than 100 and platelets less than 2,000. The kidney showed no sign of rejection at postmortem examination. Death resulted from pulmonary infection. The bone marrow was hypoplastic.

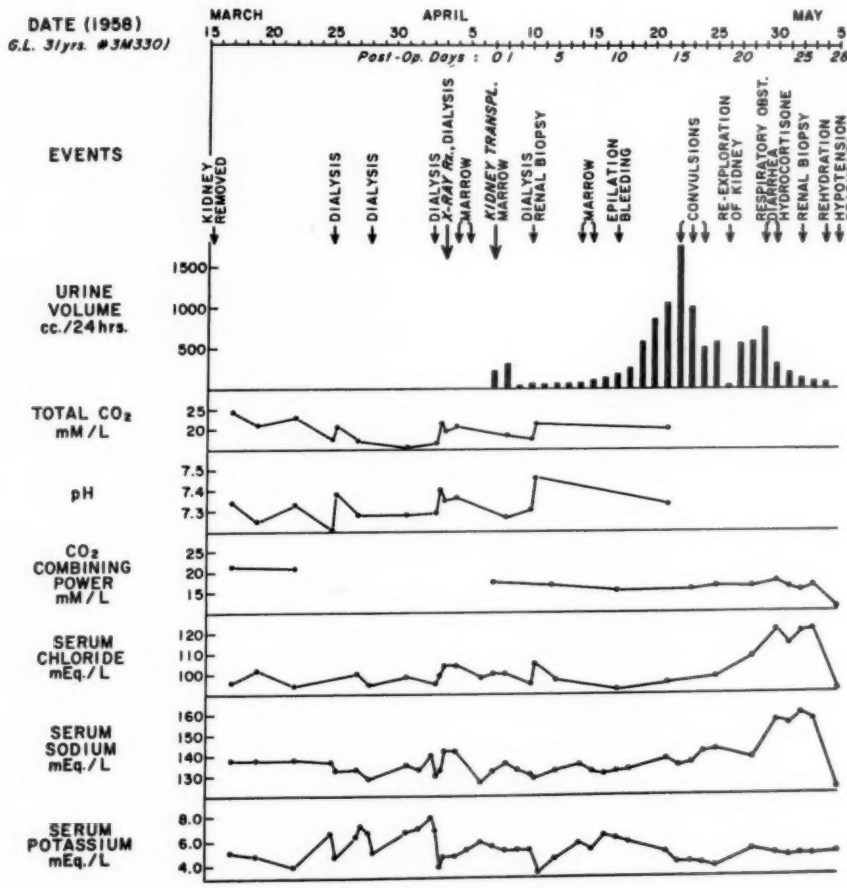

Fig. 3.—Clinical course of patient in Case. 1.

CASE 6.—G. C. was an 18-year-old girl with chronic glomerulonephritis who shared the major blood group antigens with her mother, the prospective kidney donor. After the first dose of x-ray, 250 r, the white blood count, platelet count, and reticulocyte count fell markedly. Before the second dose of x-ray was given, the mother developed a bacilluria, necessitating postponement of the transplantation. While the mother was undergoing therapy, the patient died, in spite of hemodialysis, of uremia 18 days following the single dose of x-ray therapy.

*Operation performed by Dr. John Kinney.

X-RAY DOSE

In the first 2 cases, an x-ray dose which would require bone marrow replacement was selected. The 600 r figure was extrapolated from human radiation accidents and from laboratory experiments, the 700 r dose selected because of failure to secure marrow survival at the 600 r range. In both cases, at postmortem examination, sex chromatin studies indicated preservation of host marrow rather than that of donor (Fig. 5). Although the doses were too low for successful marrow survival, the adverse side effects of x-ray were apparent. Epilation appeared on the twenty-third post-x-ray day in Case 1, and the formed blood elements practically disappeared in spite of supportive fresh transfusions. Although the convulsions may have been accentuated by radiation edema of the brain, almost certainly they were initiated by the redistribution of electrolytes seen relatively frequently during the onset of diuresis. After 700 r in Case 2, the gastrointestinal tract showed ulceration and hemorrhage although the cause of death was bronchopneumonia.

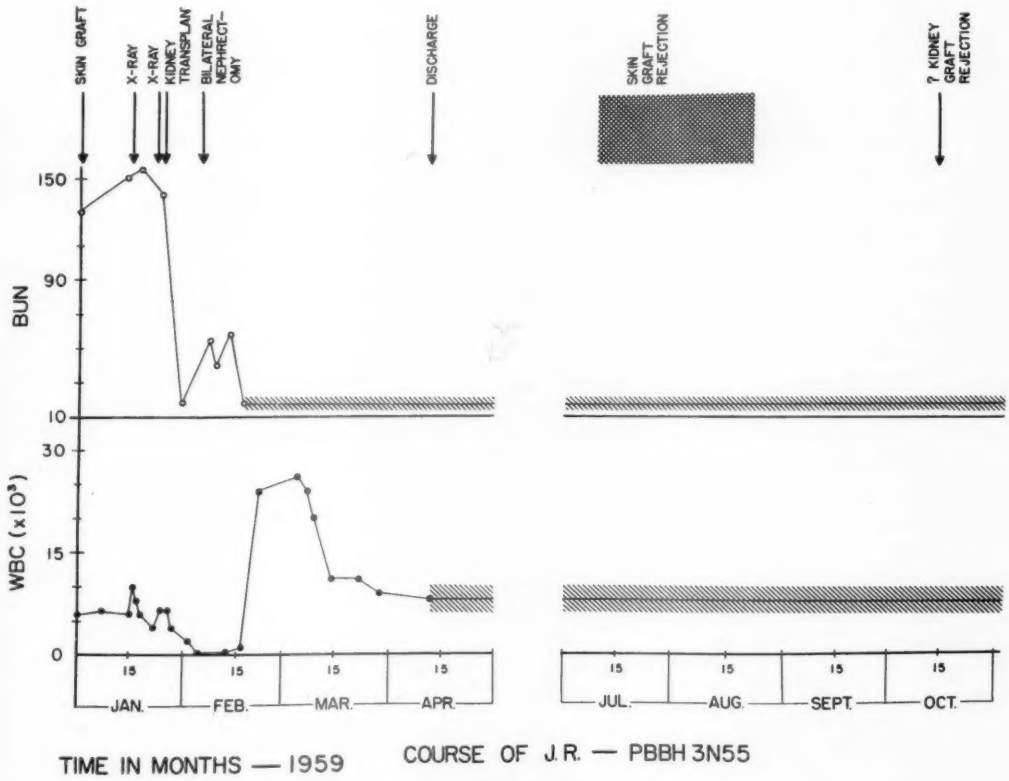

Fig. 4.—Clinical course of patient in Case 3.

Because of failure to destroy host marrow in the presence of a marked morbidity at the higher dose level, in Case 3 a lower range and a divided dose schedule were selected which might allow the host marrow to recover promptly enough to prevent the hematologic and bacteriologic morbidity. In spite of these precautions, the hematologic reaction was profound, and sepsis became a major problem. Fortunately, the marrow recovered spontaneously by the twenty-first day, in time to help combat infection. If the infused bone marrow in Case 1 had begun to proliferate and function at a similar time, the high point of the patient's course, she might also have survived.

The x-ray dose in the final 3 patients followed the pattern of Case 3. Death in Case 4 was renal in origin and unrelated to the x-ray, but in Cases 5 and 6 a marrow sensitivity to x-ray was noted. Because the leukocyte fall was more rapid and severe

after the initial dose of x-ray in Case 5, the subsequent dose was lowered to 150 r. The patient in Case 6 received only 250 r. At postmortem, 22 days and 18 days after the onset of radiation, both patients had hypoplastic bone marrow.

SOURCE OF MARROW

Ideally, the same donor would supply both marrow and kidney. Calculating from the rabbit dosage of 0.5 billion marrow cells per kilogram of body weight, the 30 to 40 billion cells required for the average human could not be procured from a single living donor within the required 72-hour period following the x-ray treatment. Multiple aspirations of marrow over a longer period of time as a possible solution to the dose problem would necessitate use of marrow preservation, in itself an undesirable procedure.[16] Therefore, in Case 1 a large pool of marrow donors was used and the marrow collected by the methods described by Wilson,[20] with the kidney donor contributing a portion of the marrow pool. In Case 2 an effort was made to follow the basic experimental design more closely, using only 1 source for marrow and kidney. Multiple fresh marrow infusions yielding 10.5 billion cells over a 7-day period were given. In neither instance did the marrow grafts survive. In later patients at a lower x-ray dose no marrow was needed, the return of the patient's marrow being equivalent to an autograft or an isogenic marrow graft.

SOURCE OF KIDNEY

In the first patient, a live kidney from a volunteer donor was not considered because of the small chance for success. If marrow survival from any 1 donor could be demonstrated, the use of that donor's kidney would have been reasonable. To test for this eventuality, skin grafts from each marrow donor and a minimum of 2 weeks' observation of the skin grafts would be necessary, obviously an impossible delay. The source of the kidney had to be available prior to the beginning of a potentially lethal course of x-ray therapy. Therefore, the irradiation was coordinated with Dr. Matson's operation. In Case 2, the mother seemed a possible donor if her skin graft and marrow graft had survived. The diovular twin donor in Case 3 seemed appropriate. There was close consanguinity plus his willingness and understanding of the entire problem. Moreover, it was known that diovular twins normally maintain reciprocal skin homografts on an average of 24 days, over twice the normal duration.[17] Prior to the irradiation, when the decision was made, the ailing twin had maintained the skin graft from the brother, the prospective donor, for over 30 days. In Case 4, in an attempt to analyze the influences of consanguinity and x-irradiation involved in successful Case 3, an unrelated child was selected as the kidney donor. Previous experience of our own (unpublished) with a neonatal donor kidney into a uremic recipient had demonstrated no prolonged survival. The same combination in an irradiated uremic recipient seemed the next logical step. A donor of the same blood group did not become available during the brief terminal illness of the patient. In Cases 5 and 6, parental donors with the same blood types as those of the patients were selected. Because of the severe x-ray effect on the hematopoietic system resulting in death from infection, insufficient data exist for clear analyses of these failures.

POSTIRRADIATION AND POSTOPERATIVE CARE

As soon as renal transplantation becomes a possibility, the patient is maintained in an isolation unit to protect against cross infection.* Patient care is defined, the premise is disinfected, exchange of bacteria is controlled, and accumulating bacteria removed in a systematized manner. A single room is selected with an ultraviolet radiation curtain across the doorway. Initial disinfection of the room, including the furniture, blankets,

*All isolation techniques were designed, implemented, and controlled by Dr. C. W. Walter and his laboratory which is supported in part by grants from Armour and Company, Fenwal Laboratories, Inc., Lehn & Fink Products Corporation, Lyman Associates, Posner and Associates, Sterling-Winthrop Research Institute, and West Chemical Products, Inc.

and walls, is performed. The bedding is sterilized by exposure to ethylene oxide, the floor by flooding with a detergent germicide. Bacterial exchange by air current can be controlled by maintenance of a positive and negative pressure within the room by means of a small fan in the window to bring sterile air into the aseptic area. A suitable filter is required to trap air-borne bacteria. An interchange area is estalished at the entrance to the isolation unit with facilities for changing into cap, mask, and gown. Tracking of bacteria can be prevented by treading on a mat of plastic foam moistened with germicide in a shallow pan located in the doorway. Hands are disinfected. X-ray machines, electrocardiograph leads, scales, and similar equipment can be disinfected by washing in an alcoholic solution of germicide. Soiled bedding and dressings are wrapped in a polyethylene or hamper bag for disposal. All personnel entering the area have their masks cultured after using.

In the 5 patients who succumbed to sepsis, the source of organism was the patient himself, rather than the environment, an effective demonstration of the efficiency of rigid simple procedures. The infections usually followed bleeding into an enclosed area which thus provided a suitable culture medium. In all patients, the chief site and source of infection was the respiratory tract, the gastrointestinal tract, or the genitourinary tract.

Except for the hazard of bleeding, the surgical care of these patients was not unduly difficult. Wound healing proceeded well in all instances. Even at the 600 r dose range, when a double pedicle flap was needed to cover the kidney in the thigh, wound healing was normal. The operative site was reopened on 3 occasions and still healed well.

MICROSCOPIC EXAMINATION

The renal homograft which was in place for 28 days in the patient receiving 600 r exhibited no rejection pattern. There was no interstitial cellular infiltration, no tubular atrophy, no arteritis, and glomeruli were normal (Figs. 6 and 7). There was neither pyroninophilia of parenchymal cells nor subendothelial cellular proliferation suggesting a reaction of the kidney itself against the host. There was moderate interstitial edema and foci of hemorrhage in the interstitial tissues and in the lumina of occasional tubules. Lymph nodes, which were grossly enlarged, had distention of the sinusoids with macrophages (Fig. 8). There were no germinal centers. The marrow was hypercellular with myelopoietic and plasma cells (Figs. 9 and 10). Many numbers of leukocytes with the female chromatin marker were noted (Fig. 5). No specific radiation-induced alterations of the cerebrum or cerebellum were apparent.

EXPERIMENTAL MATERIAL

Like man, the individual rabbit varies in its reaction to radiation. There appears to be no dose of x-ray which will destroy permanently the immune capacity of all rabbits and still allow the animal to survive. Some rabbits which received 1,100 r as a split dose at 600 r and 500 r at a 24-hour interval (lethal dose 95) have survived and respond well to a variety of antigens. If such animals receive a marrow transplant immediately after irradiation, some produce antibodies specific for the graft detectable by the indirect Coombs technique.

At sublethal doses of x-ray at 400 r (lethal dose 20) and 600 r (lethal dose 50), the depression of immunologic capacity is influenced not only by the dose of irradiation, but also by the time interval between the antigenic stimulation and the irradiation. Variation of the timing of the antigen from 24 hours following irradiation and at 7-, 14-, and 28-day intervals thereafter altered both the amount of antibody formed and the time for peak antibody production. The standard battery of test antigens included bovine gamma globulin, sheep erythrocytes, orthotopic skin grafts, and *Mycobacterium tuberculosis* in Freund's adjuvant. The most marked abrogation of the immune response occurred if the antigens were given 24 hours after irradiation. It was less marked if the antigens were given immediately after irradiation or at the 7-day interval or later. In any one animal no good correlation could be observed between the responses to the 4 antigens.[1]

Fig. 5.

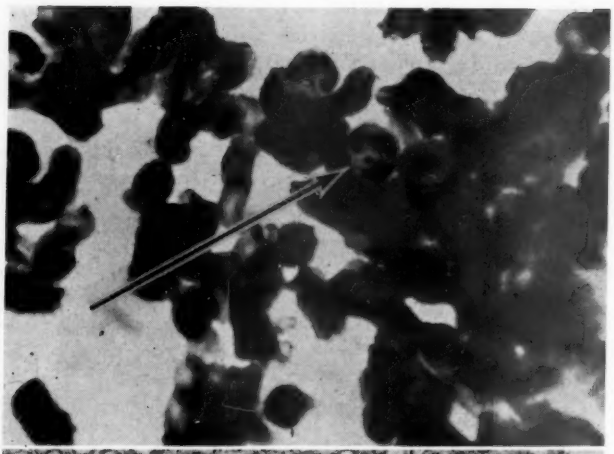

Fig. 6.

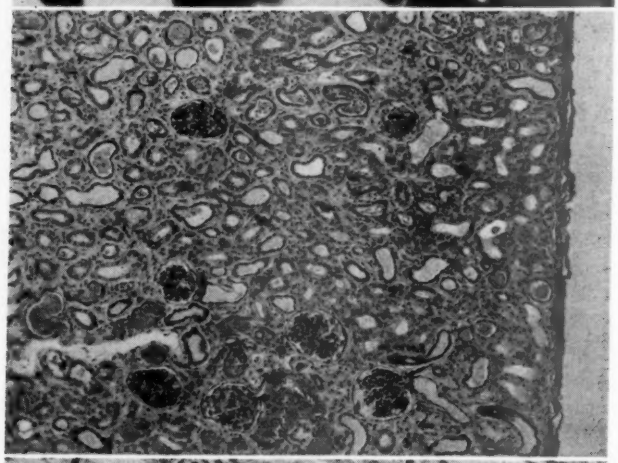

Fig. 7.

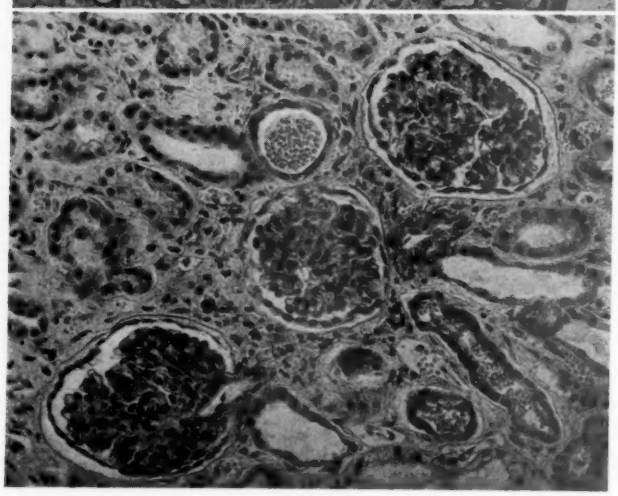

Fig. 5.—Case 1. Vertebral marrow, stained film to show leukocyte with female chromatin marker. (Wright's stain; ×900.)

Fig. 6.—Case 1. Renal homograft at postmortem. Tubules present intact epithelium and patent lumina. The glomeruli are normal. There is no interstitial cellular infiltration. (Hematoxylin and eosin; ×90.)

Fig. 7.—Higher magnification of Fig. 5. There are erythrocytes in several tubules. (Hematoxylin and eosin; ×230.)

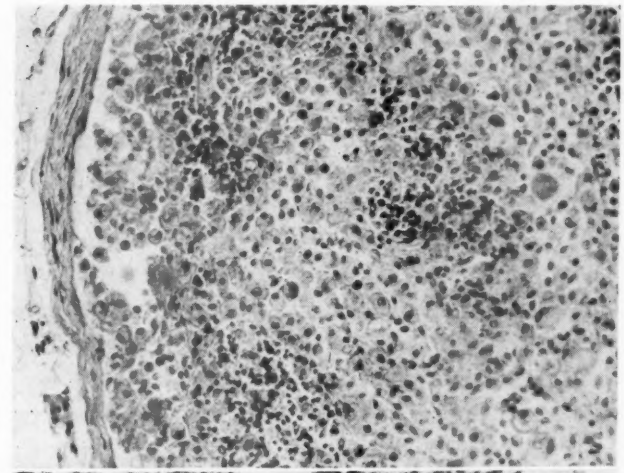

Fig. 8.

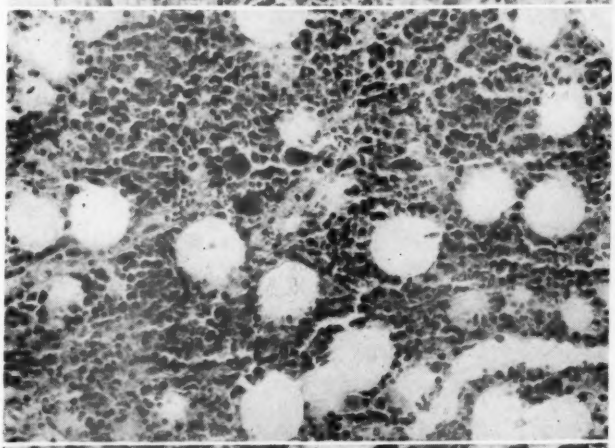

Fig. 9.

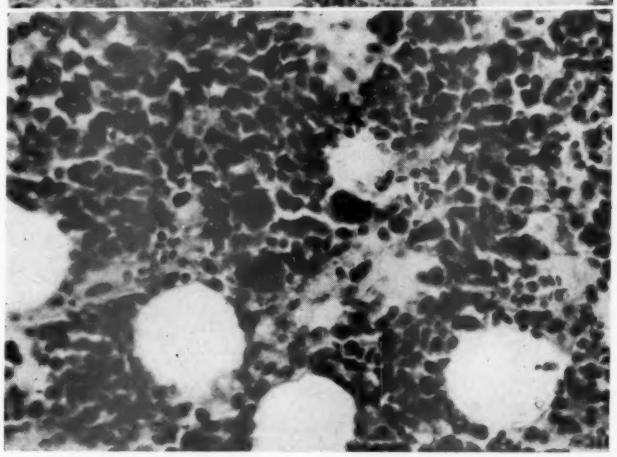

Fig. 10.

Fig. 8.—Case 1. Lymph node with peripheral and marginal sinuses distended with macrophages. Lymphocytes are few, the cluster in the right representing an atrophic follicle. (Hematoxylin and eosin; ×230.)

Fig. 9.—Case 1. Vertebral marrow. There is a cluster of megakaryocytes at the center of the field. Myelopoiesis appears active. The outlines of the fat cells are regular. Plasmocytes are present in increased numbers. (Hematoxylin and eosin; ×230.)

Fig. 10.—Higher magnification of Fig. 8. (Hematoxylin and eosin; ×400.)

DISCUSSION

The original requirements for adaptation of the experimental design of "irradiation, marrow, and homograft" to man appeared to be a heavy dose of x-irradiation to the entire host to destroy its immune mechanism, a source of hematopoietic cells capable of self-reproduction, and a subsequent graft from the marrow donor. These 3 requirements are difficult to meet in man. The dose of x-ray needed to destroy all the host's immune system is not yet determined. Because of limitations in the amount of marrow which can be obtained from a living patient at one time, the same living donor cannot give the marrow and then donate a kidney within the allotted time interval of 72 hours following irradiation. A cadaver is a prolific source of marrow, but in the absence of long-term organ perfusion to preserve viability, a cadaver kidney will of course be unsuitable. Therefore, in our first patient, marrow from several sources was used to enlarge the total dosage, and the kidney donor contributed to this pool. This concept was adapted from Hâsek,[4, 13] who produced tolerance in chicks by bone marrow and spleen cell infusions from multiple donors. The tolerance produced was not only to the skin homografts of the various donors, but also to skin donors selected at random. After failure in Case 2 at the high dose level, it was apparent that some adaptation of the experimental design to humans was needed.

The appearance of nonidentical twins, who exhibited some degree of mutual tolerance as evidenced by prolonged, reciprocal skin graft survival plus the laboratory evidence that the quantitative aspects of the immune response could be varied by alteration of the timing and of the route of antigen administration, suggested the use of a lower dose of total body irradiation. The prolonged survival of the fraternal renal transplant, now up to 12 months, coupled with the rejection of a skin graft from the same donor at 7 months, obliges us to analyze and possibly revise the basic assumptions of the experimental design. The relationship between a kidney graft anastomosed directly to its major vessel supply and a skin graft placed on the surface might suggest that skin graft rejection does not always imply subsequent kidney rejection from the same donor.[10] From experimental work, it is evident that variations of the route and the amount of antigen do produce a variation in the response following total body irradiation.[1] It was also known that a smaller dose of x-ray is required to produce successful homotransplants between lower animals who are more closely related than between those distantly related.[14]

The study of a kidney homograft in Case 1 which had been in place for 28 days gave no evidence of a "graft versus host" reaction, a possibility originally raised by Simonsen[18] and Dempster[3] on the basis of their independent work on renal transplantation in dogs. In our patient the kidney graft had ample time to produce some reaction in the defenseless host, yet there is no indication of graft reticuloendothelial activity directed against the host, suggesting perhaps that the problem of "graft versus host" reaction in human renal homotransplantation may not prove significant.

SUMMARY

An analysis of 6 patients subjected to total body irradiation followed by bone marrow, kidney, or skin homotransplantation has been made. Two patients received marrow-repressing doses of x-ray, 600 r and 700 r respectively. Four received a non-marrow-repressing dose ranging from 450 r in 2 doses to 250 r in a single dose. Bone marrow was transplanted from multiple donors, including the kidney donor in one instance, and from a single maternal donor in another. Kidney transplantation was performed on 4 occasions, twice from an unrelated neonatal donor, once from a diovular twin, and once from a father. Skin transplants were used as test grafts in 3 instances. Of all the attempts the only permanently successful homograft was of a kidney from the diovular twin to his brother following a split dose of total body x-irradiation of 450 r. Supporting laboratory studies have been used in an attempt to analyze the clinical courses.

REFERENCES

1. Brooke, M. S., Govaerts, A., and Murray, J. E.: The Immunology of Sublethally X-Irradiated Rabbits, S. Forum **10**: 853, 1959.
2. Dempster, W. J., Lennox, B., and Boog, J. W.: Prolongation of Survival of Skin Homotransplants in the Rabbit by Irradiation of the Host, Brit. J. Exper. Path. **31**: 670, 1950.
3. Dempster, W. J.: Kidney Homotransplantation, Brit. J. Surg. **40**: 447, 1953.
4. Hasek, M., and Haskova, V.: A Contribution to the Significance of Individual Antigenic Specificity in Homografting, Transplantation Bull. **5**: 69, 1958 (Abst.).
5. Hektoen, L.: The Influence of X-ray in the Production of Antibodies, J. Infect. Dis. **17**: 415, 1915.
6. Jacobsen, L. O.: Evidence for a Homograft Factor (or Factors) Concerned in the Recovery From Radiation Injury: A Review, Cancer Res. **12**: 315, 1952.
7. Krebs, C., Rask-Nielson, H. C., and Wagner, A.: Origin of Lymphosarcomatosis and Its Relation to Other Forms of Leucosis in White Mice, Acta radiol. Suppl. **10**: 1, 1930.
8. Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E.: Modification of Irradiation Injury in Mice and Guinea Pigs by Bone Marrow Injection, J. Nat. Cancer Inst. **12**: 197, 1951.
9. Main, J. M., and Prehn, R. T.: Successful Skin Homografts After the Administration of High Dosage X-irradiation and Homologous Bone Marrow, J. Nat. Cancer Inst. **15**: 1023, 1955.
10. Merrill, J. P., Murray, J. E., Harrison, J. H., Dammin, G. J., Dealy, J. B., and Friedman, E. A.: Human Kidney Transplantation Between Non-Identical Twins, New England J. Med. (in press).
11. Merrill, J. P., Murray, J. E., Harrison, J. H., and Guild, W. R.: Successful Homotransplantation of the Human Kidney Between Identical Twins, J. A. M. A. **160**: 277, 1956.
12. Murray, J. E., Merrill, J. P., and Harrison, J. H.: Kidney Transplantation Between Seven Pairs of Identical Twins, Ann. Surg. **148**: 343, 1958.
13. Murray, J. E., Wilson, R. E., Dealy, J. B., Sadowski, N., and Corsen, J.: Fate of Skin Grafts in Irradiated Rabbits Treated With Marrow From Single and Multiple Donors; International Colloquium on the Biological Problems of Grafting, University of Liège, Belgium **12**: 354, 1959.
14. Odell, T. T., Jr., and Caldwell, B. C.: Transplantation of Homologous Erythropoietic Elements in Rats After Sublethal Doses of X-radiation, J. Nat. Cancer Inst. **20**: 851, 1958.
15. Porter, K. A.: Effect of Homologous Bone Marrow Injections in X-irradiated Rabbits, Brit. J. Exper. Path. **38**: 401, 1957.
16. Porter, K. A., and Murray, J. E.: Successful Homotransplantation of Rabbit Bone Marrow After Preservation in Glycerol at -70° C., Cancer Res. **18**: 117, 1958.
17. Rogers, B. O.: The Genetics of Skin Homotransplantation in the Human, Ann. New York Acad. Sc. **64**: 741, 1957.
18. Simonsen, M., Bremann, J., Gammeltaft, A., Jenson, F., and Jorgensen, K.: Biological Incompatibility in Kidney Transplantation in Dogs, Acta path. et microbiol. scandinav. **32**: 1, 1953.

19. Stavitsky, A. A.: Antibody Synthesis by Homotransplanted Cells and Tissues, J. Immunol. 79: 187, 1957.
20. Wilson, R. W.: Technique of Human Bone Marrow Procurement by Aspiration From Living Donors, New England J. Med. 261: 3, 1959.

Discussion

DR. DAVID M. HUME (Richmond, Virginia).—I wonder if Dr. Murray would not agree that one has to be a little cautious in assuming that the prolonged take of the kidney which he has described in the human has been due to the sublethal dose of radiation which the human received. At least in the dog, we find 600 r is a lethal dose of radiation and we find that kidney transplants are rejected uniformly in the dog given 600 r. They are even rejected if the dose is stepped up to 1,000 r and it is not until we get 1,200 or 1,500 r in the dog that a prolonged survival of the kidney transplant takes place. One has to use superlethal doses in the dog to have prolonged take of the kidney. I, therefore, wonder if the evidence can be accepted completely that a sublethal dose of radiation in the human has produced the prolonged survival of this kidney or if the other factors that Dr. Murray has mentioned are perhaps not more important in this case? That is, the close genetic relationship and the chance that, in the human, one is going to have an occasional prolonged survival anyway. Dr. Murray knows that in one of the cases at the Peter Bent Brigham Hospital, the kidney functioned for 6 months even without radiation.

DR. MURRAY (closing).—I would like to answer Dr. Hume's question. We do not know, of course, whether radiation had anything to do with success, although I think that it probably did. There is experimental evidence that the more closely related the individuals are, the less radiation one needs for successful homologous transplantation. Odell and Caldwell showed this in the C and D rats. They needed only sublethal doses of x-ray to get successful marrow transplantation, whereas completely disparate strains required much higher x-ray doses.

The fact that you have had to go as high as 1,200 r in the dog before getting successful marrow survival without successful kidney survival is interesting. I think there must be some advantage in inserting the antigen intravenously early after the time of irradiation. Possibly, if you would inject some marrow from the prospective kidney donor at the time, you might encourage the host to favor the kidney. Again, as you so well know, the human and the dog are not the same as far as kidney transplants are concerned, and maybe in the human we are dealing with a more favorable situation.

IN VITRO GROWTH OF MALIGNANT CELLS FROM EFFUSIONS

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THE goal of most attempts to grow malignant cells from effusions with tissue culture techniques has been the improvement of pathologic diagnosis. In such studies only temporary growth and multiplication of the tumor cells was required.

For example, Sano[13] and Hengstmann[4] cultured the cells of pleural effusions to attain a "kinetic pathology" in which the growth patterns were used to diagnose infection processes as well as malignancy. Others, such as Weitzmann,[15] Pomerat and his colleagues,[8] Coman,[1] Gedda,[2] and our group,[7] have studied the culture of cells from effusions as a part of the investigation of culture techniques.

Interestingly, some have found it difficult to culture cells from effusions whereas others apparently have made no mention of the relative ease with which cell lines can be derived from effusions. The advantages of using cells from effusions for initiating in vitro studies are several:

1. Ascites fluid can be used as a whole or part of the culture medium.
2. The tumor cells are relatively free from contamination by other cells.
3. Comparable cytologic preparations can be made from sequential samples of the effusions and compared to serial generations of cultured cells.
4. The cells removed from large volumes of ascitic fluid can be used immediately in assay studies. For example, if one is interested in assessing the relative importance of several amino acids in culture medium, accurate duplicate cell inocula can be easily obtained. This is important because large cell inocula are able to overcome slight nutritional deficits and therefore a range of cell inocula is helpful for assessing nutritional requirements. Also, the growth requirements of the original tumor cells in effusions reflect more accurately their metabolism in vitro than do those of culture-adapted cells. Several media commercially available supplemented with human, calf, fetal calf, or horse serum are excellent once cells become adapted to (transformed) growth in vitro. One of the great challenges is the ability to culture consistently all human cells—especially small numbers of cells—de novo from biopsy material, peritoneal washings, and effusions.
5. It is relatively easier to prepare ascites tumor cells for prolonged storage in tissue banks than biopsy material.
6. Our experiences indicate that tumor cells which have apparently become adapted to growing suspended in effusions are much easier to culture successfully than others. Almost every original cell sample has grown for at least a fortnight, and 12 per cent of the cultures have been maintained over 3 months.

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The latter have consistent, rapid mitotic rates and they will probably become permanent cell lines.

The disadvantages of malignant effusions as sources of cells for tissue culture are the following:

1. Effusions do have nonmalignant cells which can survive in vitro and obscure the diagnostic accuracy of the technique.

2. Tumor cells may be present in such small numbers that even concentration of the cellular elements by centrifugation will not provide enough cells for successful culture with the media presently available. Ordinarily, about 50,000 to 200,000 cells per milliliter of medium are desirable.

3. Often effusions associated with malignancy are very bloody. Some investigators have indicated that this is detrimental to cell culture, and although we have thought that it might be harmful, there has been no evidence of its interference. Nevertheless, if large numbers of erythrocytes deteriorate, potassium levels of the culture media may increase to several times that of normal plasma. Therefore, we have tried to rinse off the red cells as soon as the tumor cells adhere to the glass (24 hours). Study of the effect of deliberate addition of erythrocytes to cultures has given inconclusive results.

4. The selection of cells for culture is limited since most abdominal malignancies do not provoke ascites and even advanced tumors may not exfoliate large numbers of cells. In other instances, exfoliated cancer cells apparently are unable to survive in the peritoneal fluid. Pleural effusions rarely have large numbers of tumor cells. Ovarian carcinomas and mesotheliomas are most frequently associated with ascites and often have large numbers of tumor cells.

5. Effusions in patients with advanced malignancy are sometimes contaminated with bacteria and even fungi. Contamination has been found both with and without prior paracentesis. Contaminated cultures cannot always be protected by the addition of antibiotics.

METHODS

Vacuum bottles to which 50 to 150 mg. of heparin had been added were used to collect the effusions. The bottle was either connected to sterile tubing fitted over the hub of the needle used for paracentesis or to a plastic catheter threaded through special trocars. The fluids were transferred to 125 ml. centrifuge bottles and centrifuged at 1,000 r.p.m. for 10 minutes. A sterile wire loop is convenient for the transfer of a drop of the centrifugate to slides for confirmation of the presence of tumor cells and their approximate number. In some instances, 1 to 3 L. may have to be centrifuged in order to recover a sufficient number of cells for culture. The cells were resuspended in tissue culture media with optimal concentrations of 50,000 to 200,000 cells per milliliter. Some cultures were attempted with a few hundred tumor cells and were successful only if clumps of tumor cells were present.

Conventional T-flasks were used in most instances. One-liter Roux bottles were valuable for culturing cells with their own effusion fluid and a minimal amount of culture medium. Several successful long-term cultures resulted from this technique.

A variety of liquid culture media were used. No specific pattern of success or failure with the different media was noted. One sort of tumor cell grew well in a certain kind of medium with a particular kind of protein supplement, whereas another cell type required a different combination. Wherever possible, a spectrum of media was used for each cell sample. The most commonly used media are listed in Table I.

TABLE I

MEDIA	PROTEIN SUPPLEMENTS:
Eagle	Fetal calf serum
Parker-199	Human cord serum
Parker-1066	Calf serum
Waymouth-752	Ascites fluid
Puck-(N-16)	
GHMJ-1 to 74*	Lactalbumin hydrolysate

*Formulas devised in this laboratory.

The salt and vitamin mixtures of the respective commercial media were used with the exception that the glucose content of medium 199 was increased twofold. Various salt and vitamin formulas were used with the GHMJ media series. The most common formula is listed in Table II.

TABLE II. SALT AND VITAMINS USED WITH GHMJ-8 MEDIA

NaCl	6,600	Folinic acid	0.05
KCl	150	Inositol	5.0
$Ca(NO_3)_2 \cdot 4H_2O$	200	Lipoic acid	0.5
$MgSO_4 \cdot 7H_2O$	300	Nicotinic acid	0.1
NaHCO3	2,000	Nicotinamide	0.5
Na_2HPO_4	100	Nicotinic acid	0.5
KH_2PO_4	100	Pantothenate	0.5
Glucose	3,000	Pantothenic acid	0.1
Ascorbic acid	50	Pyridoxal PO_4	0.5
p-Aminobenzoic acid	0.1	Pyridoxine HCl	0.5
Biotin	0.5	Riboflavin	0.5
Choline	2.0	Thiamine	2.0
Cyanocobalamin	0.1	Phenol red	5.0
Folic acid	0.5		

Cultures with evidence of rapid growth were fed every 48 hours. Slow growing cultures were fed weekly by replacement of one half of the medium. Subculturing was performed by scraping the cells off the glass with a rubber policeman or platinum wire loop. A 0.25 per cent trypsin solution was used to provide single cell suspensions, but this was considered more detrimental to the cells than the mechanical removal. Gassing with 5 per cent CO_2 in compressed air did not appreciably affect cell growth if cell inocula were large but was advantageous for the culture of minimal numbers of cells.

Whenever possible, solid tumor biopsy material was obtained for culture from the same patients with effusions. In all of these instances, the cells from effusions were easier to establish in vitro than the biopsy cells. (Approximately 600 solid biopsy specimens have been cultured in the same period of time, and although some cultures survived 1 to 5 passages, none have become established lines.)

It is noteworthy that sterile effusions in the original vacuum bottles may be kept in the refrigerator for at least a week and still contain viable tumor cells. This seems true only for those tumor cells which have undergone transformation. All but one of the cell samples which survived refrigeration temperatures for 1 week also provided our long-term culture lines (Table III).

TABLE III

EFFUSIONS	VIABLE (DAYS)	NONVIABLE (DAYS)
Sk—Gall bladder	9	12, 15, 18
Ma—Ovary	7	10, 13, 15, 18
Jo—Ovary	5	—
Bl—Mesothelioma	6	15+
Da—Osteogenic sarcoma	7	—
Re—Ovary	7	20

Aliquots of effusions which were merely frozen and kept in a dry-ice box failed to survive 3 months. Cells which were added to 10 per cent glycerin and kept at −70° C. have remained viable many months.[3]

RESULTS

Table IV lists the effusions which have been cultured. It should be noted that in some instances multiple cultures of a single ascites sample were made or, if available, additional ascitic fluid was removed from the patient and cultured over periods of days and weeks. In most instances, the cells of a sample either grew profusely in several media or, on the contrary, grew sparsely. Usually one medium provided optimal cell growth and cells free of a granulated appearance. Lactalbumin hydrolysate (0.5 per cent) in Hank's balanced salt solution supplemented with 10 per cent fetal calf serum was the best "universal" medium.

TABLE IV

TUMOR	NUMBER OF SPECIMENS	PAPANICOLAOU CYTOLOGY (POSITIVE)	SUCCESS CULTURE 10 DAYS	CULTURE OVER 3 MONTHS	ACTIVELY GROWING
Ovary	14	13	14	10	3
Stomach	9	8	9	3	0
Colon	7	5	7	2	0
Breast	5	4	5	2	0
*Miscellaneous	14	12	14	6	3
Totals	49	42	49	23	6

*Gall bladder; mesothelioma, osteogenic sarcoma.

It is evident that short-term culture of malignant cells from effusions was very successful. Nevertheless, the gradual dying-out of most primary cell cultures (even with an initial burst of accelerated growth) occurred in 3 to 10 weeks. Presumably, the culture medium still was unable to provide all of the nutritional needs and proper physical environment for the cells.

Twenty-three cultures survived 3 months or longer but a majority subsequently developed a decreasing growth rate which could not be reversed by subculturing or changing the kind of medium or patterns of refeeding. Often both subcultures and the original culture died out simultaneously. In other instances, cultures with the greatest number of cells in the original inoculum seemed to maintain themselves longer—as if some critical intracellular growth requirement became exhausted and was not replaced by the medium.

The transformation of original cell cultures to a condition in which the cells assume a consistent heightened growth rate is not always obvious. For example, culture No. 1270, which originated from a malignant mesothelioma, had a generation time of about 20 hours. This growth rate has remained more or less uniform for 1 year.

In contrast, culture No. 1309 which was originated from ascites in association with a carcinoma of the gall bladder had an initial rapid growth which gradually became less prolific over a period of 8 weeks. Suddenly a colony of epithelium-like cells developed in 1 of 3 culture lines. These transformed cells have been maintained for 20 passages. One must be careful that such a sudden change in the cells has not resulted from accidental contamination by cells from

some other cell line being maintained in the laboratory or from mislabeling. One such accident occurred in this laboratory. In most instances, however, the strains have definite cytologic and nutritional differences which permit recognition. These features are not completely reliable since the cells may assume various morphologic configurations, depending upon the media and protein supplements being used. (Further, most tumor cell lines are a mixture of slightly different cells; for example, cells with different chromosone numbers.) This becomes evident if clones are made from the cultures and the variations in cytology, histochemical tests, and nutritional requirements are restudied.

DISCUSSION

The relative ease with which human tumor cells from effusions can be cultured is not paralleled by the successful culture of many animal ascites tumors. One would think that the Ehrlich ascites cell would certainly be well adapted for in vitro culture, but such is not the case. Several investigators have recorded their inability to culture successfully the several Ehrlich cell lines. A series of special media have been tried in this laboratory without obtaining more than temporary growth. Limited growth of these cells in suspension cultures has been reported.

Cells of effusions in which counts as high as 1 to 10 million cells per milliliter are easier to culture, in most instances, than those from effusions in which cell counts are less than 1,000 cells per milliliter. Even a small inocula of the former will grow readily, suggesting that these cells have already undergone important adaptive changes in vivo. In most instances, the cell count of effusions is less than 1,000 cells per milliliter and large volumes of fluid must be centrifuged in order to obtain sufficient numbers of cells for culture. These cells are probably merely exfoliated cells which are surviving but not actively multiplying in the fluid. This may explain why their growth in vitro is as difficult as the long-term culture of cells from biopsy material.

Study of the mitotic rates of ascitic cells should be revealing. Certainly the reaccumulation of ascites is not correlated with the number of cells in suspension and therefore is not an indication of probably successful growth of the cells in vitro. The role of the cell-free ascites fluid as a nutrient medium should not be totally depreciated. In some instances ascites cells have grown well in a medium made up of 50 per cent or more of ascites fluid, but the growth rate is usually less than that of comparable cultures in serum-containing media.

Weitzmann[15] is said to have reported in 1938 the first 2 cultures of malignant cells from patients with pleural and peritoneal carcinomatosis, respectively.

Coman[1] cultured ascitic and pleural fluid from 23 patients (9 had malignancy) in order to confirm the viability and growth potential of the suspended cancer cells. A roller tube technique was used. The packed cells were held in a plasma clot and bathed with a medium consisting of a balanced salt solution and human fetal serum. Apparently all 5 cultures of ascitic fluid containing cancer cells grew and survived for many weeks. Similarly, fibrocytic cells grew from the pleural fluid of 2 patients with fibrosarcoma, and epithelial

cells were cultured from a patient with metastatic carcinoma. In contrast, cells from a leukemic effusion failed to survive beyond 2 weeks.

Ivers and Pomerat[5] described the failure to find malignant cells in cultures of ascitic fluid from a patient with advanced gastric cancer, and successful culture of malignant cells from the ascites of 2 patients with ovarian carcinoma.

Subsequently, Pomerat, Nowinski, and Rose[8] detailed the advantages of ascitic fluid as a culture medium.

The most complete paper concerned with the culture of human effusions is that by Sano in 1953.[13] He cultured pleural effusions from 37 patients, peritoneal effusions from 8 patients, pericardial fluid from 3 patients, and 1 joint fluid specimen. Both autogenous plasma plus mouse embryo extract and chicken plasma plus chicken embryo extract were used as media. Both roller tubes and double coverslip chambers were employed. Of 17 patients with presumed or confirmed diagnoses of bronchogenic carcinoma or metastatic carcinoma from a distant site, tissue culture growth of tumor cells was evidenced in 12 and negative in 5. The culture of malignant cells had approximately the same accuracy as examination of Papanicolaou smears. Of 5 peritoneal ascitic fluid specimens all but 1 were successfully cultured. The author concluded that tissue culture had merit as an adjunct diagnostic procedure.

Romeo and Ferri[11] studied the cultures from 29 patients with peritoneal carcinomatosis. The cells were recovered by centrifugation of the ascitic fluid. The culture medium consisted of Hank's salt mixture, 20 per cent heat-inactivated human serum, 0.5 to 1.0 per cent lactalbumin hydrolysate, and antibiotics. Unfortunately, the cultures could not be kept growing beyond 22 days and none survived 1 month. In direct contrast are the reports of Pulvertaft and Weiss,[10] who have recently reviewed their experiences in examining about 500 effusions containing malignant cells and their culture. They state that they were not able to establish permanent strains of malignant cells in 200 attempts. It was concluded that this failure may reflect a diminished viability of the cells in effusions.

This conclusion is contrary to our experience and that of most investigators. Indeed, the primary reason for publishing this paper is to remind investigators of the comparative ease with which abundant initial growth can be obtained from malignant cells from effusions, and the high proportion of permanent strains which can be obtained.

It seems reasonable to theorize that tumor cells in some effusions have undergone important adaptive changes in addition to gaining the ability to grow suspended in a body-fluid medium. This probably involves an enormous number of changes in addition to measurable known factors such as pH, glucose levels, relative anoxia, and others. The importance of determining these factors is evident when one considers the need for finding more effective ways of selectively destroying cancer cells in the body cavities. For example, ovarian cancers grow almost exclusively in the peritoneal cavity but are rarely cured by surgery or x-ray.

A minimal number of cells per unit volume of culture medium seems necessary for the growth of most cells. Cell-to-cell associations apparently permit

interreactions of physical and chemical conditions which prolong survival. This mutual dependence of cells has defied analysis. It would seem that the tumor cells in effusions may have overcome the inhibitory effect of being relatively remote from neighboring cells. A majority of cells in effusions are not "adapted" and may grow abundantly, temporarily, then slowly and die out in 4 to 8 weeks. Perhaps this is most easily explained (but not necessarily correctly) by the suggestion that the cells carry critical growth factors bound to the cell envelope with them which are not replaceable in vitro. The factors may be replaceable in vivo as a result of a continual association of the ascitic fluid with the living body.

The successful growth of one or a few hundred cells in vitro has been the subject of many studies. Sanford, Earle, and Likely[12] were able to grow single cells by using "conditioned" media from cultures of the same sort of cells. Subsequently, single cells were grown in capillary tubes. Apparently, the favorable cell-medium volume ratio prevented a loss of the cell products which are necessary if the cells are to maintain themselves. Weiss and others have studied the mutual dependence of cells for many years.[14]

Klein and Klein[6] have investigated the conversion of solid tumors to the ascites form and have indicated that this process involves nutritional changes and the development of independence from the growth factors released by adjacent cells. For example, an inoculum of a small number of cells may result only in solid tumor growth, whereas an inoculum of a million cells or more apparently provides sufficient mutual cell support so that further growth in suspension can take place. Finally, permanent "adaptation" to an ascitic form may occur. Then a few cells or even one suffices for establishing ascitic growth. The study of cytologic and nutritional aspects of these cells has been reviewed by these investigators.

Another technique for the original culturing of a minimal number of cells employs layering of the fresh cells over irradiated cells, as originally described by Puck and his colleagues.[9] In 1955 they demonstrated the supportive activity of irradiated "feeder cells" upon the survival and multiplication of single mammalian cells. We have successfully used this technique for the culture of tumor cells from the blood and from peritoneal washings in a few instances.[7]

Several cell-rich effusions were diluted with culture medium and used directly as suspension cultures. Although temporary growth has occurred for several days, it was followed by a period in which no multiplication took place and finally, after about 10 days, a sustained decrease of the cell population. Cell samples obtained from the spinner culture during the latter period often had a granular appearance but still grew well when transferred to T-flasks. A colleague, Mr. David Mount, has suggested the use of dialysis equipment for the maintenance of suitable conditions in spinner cultures and for continuous analyses of the changes in the medium associated with cell growth. The use of suspension cultures has been reviewed by others and will not be discussed here.

The purposes of continuing in vitro culture of human cancer cells range from its practical use as a diagnostic technique to, simply, the basic challenge of our inability to grow various kinds of human cells consistently, particularly in defined media. Study of the nutritional requirements of initial cell cultures,

their response to chemotherapeutic agents, the growth of gram to kilogram amounts of malignant cells for biologic, chemical, and immunologic studies, evaluation of cell physiology, hormone production, and so on, and intensive study of the cell-to-cell relationships which may reveal mechanisms of cell growth regulation, besides many other studies of human cells, await further development of tissue culture techniques.

SUMMARY

1. The cells recovered from 49 peritoneal cavity effusions from patients with malignancy have been cultured in vitro. Temporary growth occurred in all instances, was sustained over 13 months by 23 cultures, and 6 apparently permanent cell lines have been established.

2. Cells from effusions with the highest cell concentrations are easiest to grow and most apt to become stable culture lines. Such cells have probably already undergone adaptation and are actively multiplying in contrast to the cells which have merely exfoliated into the body cavity.

3. The advantages of culturing cells from malignant effusions include the ease of culture, relative freedom of contamination of tumor cells from other tissue cells, and more or less adaptation to growth in a fluid medium. The latter affords the opportunity of using some of them as original suspension cultures and/or for assay purposes.

REFERENCES

1. Coman, D. R.: Human Neoplasms in Tissue Culture. Observations Upon Cells Derived From Peritoneal and Pleural Effusions, Cancer Res. **3**: 526-530, 1943.
2. Gedda, L.: Contributo alla diagnosi dei versamenti neoplastici mediante la coltura *in vitro* Cancro **3**: 126, 1932.
3. Hauschka, T. S., Mitchell, J. T., and Niederpruem, D. J.: A Reliable Frozen Tissue Bank: Viability and Stability of 82 Neoplastic and Normal Cell Types After Prolonged Storage at 78° C., Cancer Res. **19**: 643-653, 1959.
4. Hengstmann, H.: Zur cytologischen Diagnostik von carcinomatösen Pleura- und Peritonealergüssen durch Zellkulturen, Ztschr. f. klin. Med. **139**: 58-66, 1941.
5. Ivers, J. B., and Pomerat, C. M.: Observations on Cells From Ascitic Fluid Cultures *In Vitro*, Texas Rep. Biol. & Med. **5**: 92-111, 1947.
6. Klein, G., and Klein, E.: The Evolution of Independence From Specific Growth Stimulation and Inhibition in Mammalian Tumor-Cell Populations, Sympos. Soc. Exper. Biol. **11**: 305-328, 1957.
7. Moore, G. E., Mount, D. T., and Wendt, A. C.: The Growth of Human Tumor Cells in Tissue Culture, S. Forum **9**: 572-576, 1958.
8. Pomerat, C. M., Nowinski, W., and Rose, G. G.: Tissue Cultures of Cells From Body Fluids, Texas Rep. Biol. & Med. **8**: 521-531, 1950.
9. Puck, T. T., and Marcus, P. I.: A Rapid Method for Viable Cell Titration and Clone Production With Hela Cells in Tissue Culture: The Use of X-irradiated Cells to Supply Conditioning Factors, Proc. Nat. Acad. Sc. **41**: 432-437, 1955.
10. Pulvertaft, R. J. V., and Weiss, L.: The Identification of Living Malignant Cells in Exudates, J. Clin. Path. **10**: 390-393, 1957.
11. Romeo, V., and Ferri, L.: Modern Methods for Cytological Diagnosis of Pleural and Peritoneal Effusions, Sci. Med. Ital. **6**: 68-108, 1957.
12. Sanford, K. K., Earle, W. R., and Likely, G. D.: The Growth *In Vitro* of Single Isolated Tissue Cells, J. Nat. Cancer Inst. **9**: 229, 1948.
13. Sano, M. E.: The Diagnostic Value of Tissue Culture Studies of Pleural Effusions, Surg. Gynec. & Obst. **97**: 665-676, 1953.
14. Weiss, P.: Prospectives in the Field of Morphogenesis, Quart. Rev. Biol. **25**: 177, 1950.
15. Weitzmann, G.: Die Züchtung von Kulturen aus menschlichen Pleura- und Peritonealergüssen, Arch. f. exper. Zellforsch. **22**: 414-421, 1939.

Review of Recent Meeting

MEETING OF THE SOCIETY OF UNIVERSITY OF SURGEONS

FEBRUARY 11-13, 1960, MINNEAPOLIS, MINNESOTA

RALPH A. DETERLING, JR., M.D., BOSTON, MASS.

The twenty-first annual meeting of the Society of University Surgeons was held in Minneapolis, Minnesota, on Feb. 11-13, 1960, where the Society was the guest of the University of Minnesota Medical Center.

On Thursday morning, February 11, an impressively large group of members and guests assembled in the Mayo Memorial Auditorium on the University of Minnesota campus. As usual, the morning session was devoted to papers by the host Department of Surgery, with Dr. Richard L. Varco and Dr. Donald J. Ferguson presiding, following introductory remarks by Dean Robert B. Howard and Dr. Owen H. Wangensteen, Professor and Chairman of the Department of Surgery. The following papers were presented:

1. The Mode of Action of Staphylococcus Alpha Toxin. Alan P. Thal, M.D.
2. Experimental Wound Infections—Contamination Versus Surgical Technique. John D. Condie, M.D., Robert E. Manion, B.S., and Donald J. Ferguson, M.D. (Veterans Hospital, Minneapolis).
3. Acute Necrotising Pancreatitis by Intermittent Ischemia. Ward O. Griffin, M.D. and Demetre Nicoloff, M.D.
4. The Role of the Jejunum in Gastric Hypersecretion Following Porto-Caval Shunt. Aldo Castaneda, M.D.
5. Diagnosis of Mesenteric Infarction (An Experimental Study). Karel B. Absolon, M.D., Sister Victorine Long, M.S., and Samuel W. Hunter, M.D. (St. Joseph's Hospital, St. Paul).
6. Factors Regulating the Excretion of Aldosterone. M. Ameli, M.D., W. Moran, Jr., M.D., T. Grage, M.D., and B. Zimmermann, M.D., Ph.D.
7. Evaluation of a Double Isotope Derivative Method for Estimation of Aldosterone and Its Application to Adrenal Vein Plasma. W. H. Moran, Jr., M.D., and Audrey Martinson, B.S.
8. Is Acquired Tolerance a Type of Immunological Paralysis? J. Bradley Aust, M.D., Ph.D.
9. Prolongation of Skin Homograft Survival by Dexamethasone in Laboratory Animals. William R. Meeker, M.D., Stanley Gall, B.S., and Richard L. Varco, M.D., Ph.D.
10. Evaluation of Autologous Spleen and Bone Marrow to Protective Agents Following Lethal Total Body Irradiation in the Dog. Donald L. Lamb, M.D., William D. Kelly, M.D., Ph.D., and John Condie, M.D. (Veterans Hospital, Minneapolis).
11. Studies in Isolated Perfusion. Robert K. Ausman, M.D., and J. Bradley Aust, M.D., Ph.D.
12. Selective Intra-arterial Perfusion of Total Peritoneal Viscera for Chemotherapy of Abdominal Carcinomatosis. Claude R. Hitchcock, M.D., Ph.D., John Bascom, M.D., Richard Shepard, M.D., and Gilbert F. Mueller, M.D. (Minneapolis General Hospital).
13. Effects of Certain Subcortical Brain Lesions on Induced Tremor. R. H. Strassburger, M.D. and Lyle A. French, M.D., Ph.D.

Following an excellent luncheon at the Coffman Memorial Union, the remainder of the local program was presented, with C. W. Lillehei presiding.

14. Microcirculation and Splanchno-Hepatic and Renal Blood Flow Studies During Prolonged Total Cardiopulmonary Bypass. David M. Long, Jr., M.D., Robert D. Sellers, M.D., Prem Buri, M.D., John McKelvey, A.B., and C. Walton Lillehei, M.D., Ph.D.
15. The Relative Effect of Chronic Ischemia and a Myocardial Revascularization Procedure on the Ventricular Fibrillation Threshold. Lloyd D. MacLean, M.D., Ph.D., and Clifford M. Phibbs, M.D. (Ancker Hospital, St. Paul).
16. Gas Embolism in Extracorporeally Oxygenated Blood. David E. Donald, M.D., James C. Fellows, M.D., and John W. Kirklin, M.D., Ph.D. (Mayo Clinic, Rochester).
17. The Relationship of the Sympatho-adrenal System to Potassium Flux and Cardiac Irritability Induced by Alterations in Blood pH. Bernard Goott, M.D., J. Rosenberg, M.D., R. C. Lillehei, M.D., and Fletcher A. Miller, M.D., Ph.D.
18. Direct Measurement of the Altered Response in Coronary Flow After Epinephrine During Respiratory Acidosis. E. M. Berkas, M.D., N. Trotman, M.D., and Donald J. Ferguson, M.D. (Veterans Hospital, Minneapolis).
19. Potassium Flux in the Isolated Perfused Rabbit Heart. E. W. Humphrey, M.D. and J. A. Johnson, M.D. (Veterans Hospital, Minneapolis).
20. Significance of Changes in Serum Serotonin and Plasma Epinephrine and Norepinephrine During Endotoxin Shock. J. C. Rosenberg, M.D., R. C. Lillehei, M.D., and Walter H. Moran, Jr., M.D.
21. A Naturally Occurring Lipid That Neutralizes Heparin Activity in Blood. Donald S. Amatuzio, M.D., Francisco Grande, M.D., and Lyle J. Hay, M.D., Ph.D. (Mt. Sinai Hospital, Minneapolis).
22. Evaluation of Local Measures in Treatment of Snake Bite. Po Myat Ya, M.D., and John F. Perry, M.D., Ph.D.

Once again a provocative and most informative session on surgical education was presented with Dr. J. Englebert Dunphy as presiding officer. Elaborating on some trends in the education of the surgeon in training, Dr. Wangensteen traced the growth of research in his own department. Dr. Henry Harkins was unfortunately delayed by weather conditions and could not present his paper on the growth of research in the surgical training program. The potential mutual value of mental and corrective institutions and university surgical training programs was explored effectively by Dr. John A. Schilling and Dr. Bernard Zimmermann.

A well-attended and very enjoyable cocktail party and dinner dance were held in the Hall of States of the Leamington Hotel that evening.

The Scientific Program presented by members of the Society on February 12 and 13 is published in this issue of SURGERY.